TUBEROUS SCLEROSIS-ASSOCIATED ENAMEL PITTING AND GINGIVAL FIBROMAS: FAMILIAL VS. SPORADIC DISEASE; GENOTYPE-PHENOTYPE CORRELATIONS

by

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INTRODUCTION

Tuberous sclerosis is an inherited disorder with a gene frequency estimated to be as high as 1 in 6,000 to 1 in 10,000.[1] Affected individuals have a wide variety of clinical symptoms usually involving benign tumor growths (hamartomas) of different parts of the body. The brain, heart and kidney are most frequently affected with a wide variability in the expression of this disorder. Individuals may carry the affected gene and have few or no clinical symptoms. Other individuals may be subject to seizures, autism, mental retardation, congenital heart disease, kidney tumors, renal failure, hypertension, skin lesions, and pitting of enamel as well as oral fibromas.[2] There are two genes that are associated with tuberous sclerosis: TSC1 on chromosome 9p34, and TSC2 on chromosome 16p13.3.[3] Two-thirds of TSC cases are sporadic and are thought to represent new mutations with the majority being TSC2. The remainder are familial with an equal distribution between TSC1 and TSC2, and these patients tend to be less severely affected clinically than individuals with sporadic TSC.[4] While many people with TSC are thought to have spontaneous mutations, the genes are also known to exhibit extremely variable expression. Even though penetrance is very high, there are often individuals with some of the manifestations of TSC but insufficient involvement to make a definitive diagnosis. It is unknown why there is such variability in the symptoms expressed by the people who carry one of these mutations.

The TSC2 gene was cloned in 1993 and the TSC1 gene cloned in 1997.[5] The full genomic sequence of both genes is now currently available with the TSC2 gene being significantly more complex than the TSC1 gene. Thus far, over 148 unique mutations in

TSC1 and 251 unique mutations in TSC2 have been identified. Clinically it is not possible to distinguish patients with TSC1 from patients with TSC2, and there have been only preliminary genotype-phenotype correlations established.

There have been many reports of the oral manifestations of tuberous sclerosis. It is clearly established that affected individuals demonstrate enamel pitting and fibromas of the oral tissues at a frequency greater than that of the population as a whole. There have been no studies that have compared the oral findings of enamel pitting and oral fibromas in familial and sporadic TSC, or that have looked at genotype-phenotype correlations as related to oral findings. Additionally, there have been no reports relating these oral findings in TSC patients to other disease variables (e.g. IQ, seizure activity, skin angiofibromas, cardiac involvement, and renal disease).

The purpose of this investigation was:

1. To study the incidence and severity of enamel pitting and gingival fibromas in patients with a confirmed diagnosis of TSC.

2. To compare the incidence and severity of enamel pitting and incidence of oral fibromas in sporadic vs. familial cases of TSC.

3. To correlate the incidence of enamel pitting and oral fibromas in patients with TSC2 to the following groups of mutations:

    a. Truncating mutations (small insertions and deletions, nonsense, splice site) and large genomic deletions and rearrangements.

    b. Nontruncating mutations (missense point mutations and in-frame deletions).

4. To correlate the incidence and severity of enamel pitting and incidence of oral fibromas in individuals with TSC to phenotype severity as measured by intellectual ability, seizure activity, skin involvement, cardiac involvement, retinal lesions and kidney disease.

The hypothesis is that the incidence and severity of enamel pitting and the incidence of gingival fibromas in individuals with TSC sporadic disease is greater than in individuals with TSC familial disease. Additionally, the incidence and severity of these oral findings is related to phenotype severity and may be associated with specific mutation types.

REVIEW OF LITERATURE

EPIDEMIOLOGY AND HISTORY OF TUBEROUS SCLEROSIS COMPLEX

Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder and is included in a group of syndromes known as the neurocutaneous syndromes, neurodermatoses, ectodermoses, or phakomatoses.[6] Other major disorders in this group include von Recklinghausen's generalized neurofibromatosis, and Sturge Weber syndrome. TSC-affected individuals have a wide variety of clinical symptoms usually involving hamartomas of different parts of the body. A hamartoma is defined as "a focal malformation that resembles a neoplasm, grossly or even microscopically, but results from faulty development in an organ; composed of an abnormal mixture of tissue elements, or an abnormal proportion of a single element, normally present in that site which develop and grow at virtually the same rate as normal components, and are not likely to result in compression of adjacent tissue."[7] Unlike other neurocutaneous diseases, hamartomas can develop in every organ system of the body but are especially prevalent in the brain, eyes, kidneys, heart, and skin.[2,8] The lungs, skeleton, endocrine glands, and the tissues of the oral cavity are occasionally affected.[3,9] Hamartomas involving the skeletal muscles have not been reported.

The incidence of TSC is estimated to be between 1 in 6000 to 1 in 10,000 births.[10] The clinical expression is extremely variable and unpredictable; therefore, the frequency of diagnosed cases is likely to under represent true prevalence.[10] Both sexes are affected equally with two-thirds of cases representing spontaneous mutations. Penetrance is

estimated to be approximately 95 percent; however, there have been reported cases where neither parent was apparently affected. These cases, after rigorous clinical evaluation and molecular genetic analysis, have recently been disproved.[4,5] It is likely that the reported high spontaneous mutation rate may in fact represent the variability in expression of the gene and the associated difficulty in diagnosis of the disease.

Delineating the full extent of TSC involved a multidisciplinary effort, with contributions from pathologists, neurologists, dermatologists, geneticists, radiologists, and dentists of the past and present century.[11] Von Recklinghausen described a neonate with characteristics of tuberous sclerosis in 1862[12]; however, the name tuberous sclerosis was introduced by Bourneville in 1880 after reporting on a three-year-old patient with mental retardation, seizures and facial angiofibromas.[8,13] He described the characteristic brain lesions (hamartomas) as potato or root-like in consistency, hence the name tuber. Tubers are regions of disorganized cortical lamination containing heterogeneous cellular elements including abnormal and normal appearing neurons, astrocytes and giant cells. Adenoma sebaceum facial lesions (angiofibromas) and the "Shagreen" patches were initially described by Pringle in 1890. Vogt, in 1908, proposed the "classic triad" of seizures, mental retardation, and adenoma sebaceum (facial angiofibromas) and noted that cardiac and renal tumors were part of the disease.[11] This "triad" of symptoms has been frequently cited in the literature as diagnostic for TSC but is misleading. Recent studies have shown that the full triad is evident in only about one-third of patients.[14,15]

The diagnosis of TSC is dependent on a thorough clinical examination of cutaneous features along with cranial computed tomography, magnetic resonance imaging, echocardiography and renal ultrasound. As with other neurogenetic disorders, a

definitive diagnosis will ultimately rest on demonstration of specific genetic abnormalities, in addition to clinical features.[2] Identification of genetic mutations in TSC, however, remains limited to research applications, so that diagnosis continues to rest on recognition of characteristic clinical signs and symptoms.[2] TSC diagnostic criteria was initially established by Dr. Manuel R. Gomez and was used for many years. The most recent diagnostic criteria for tuberous sclerosis was developed at the Tuberous Sclerosis Consensus Conference in 1998.[16]

REVISED DIAGNOSTIC CRITERIA FOR
TUBEROUS SCLEROSIS COMPLEX

Major Features

1. Facial angiofibromas or forehead plaque.
2. Nontraumatic ungual or periungual fibroma.
3. Hypomelanotic macules (more than three).
4. Shagreen patch (connective tissue nevus).
5. Multiple retinal nodular hamartoma.
6. Cortical tuber (see "a" on next page).
7. Subependymal nodule.
8. Subependymal giant cell astrocytoma.
9. Cardiac rhabdomyoma, single or multiple.
10. Lymphangiomyomatosis (see "b" on next page).
11. Renal angiomyolipoma.

Minor Features

1. Multiple randomly distributed pits in dental enamel.
2. Hamartomatous rectal polyps (see "c" below).
3. Bone cysts (see "d" below).
4. Cerebral white matter radial migration lines (see "c, d, e" below).
5. Gingival fibromas.
6. Nonrenal hamartoma (see "c" below).
7. Retinal achromic patch.
8. “Confetti” skin lesions.
9. Multiple renal cysts (see "c" below).

a. When cerebral cortical dysplasia and cerebral white matter migration tracts occur together, they should be counted as one rather than two features of tuberous sclerosis.

b. When both lymphangiomyomatosis and renal angiomyolipomas are present, other features of tuberous sclerosis should be present before a definite diagnosis is assigned.

c. Histological confirmation is suggested.

d. Radiographic confirmation is sufficient.

e. One panel member at the 1998 conference felt strongly that three or more radial migration lines should constitute a major sign.

MAKING THE DIAGNOSIS

Definite TSC: Either two major features or one major feature plus two minor features.

Probable TSC: One major plus one minor feature.

Possible TSC: Either one major feature or two or more minor features.

SYSTEMIC PHYSICAL FINDINGS

Pathologically, tuberous sclerosis is a disorder of cellular migration, proliferation, and differentiation.[17] TSC affects different organ systems in various ways and many of the manifestations are age dependent. The disorder results in lesions including subependymal brain nodules and calcifications, astrocytomas, retinal phacomas, sclerotic bone lesions, hamartomas or cystic lesions of the brain, heart, lungs, kidneys, spleen, liver, uterus, and soft tissues, and dental enamel pitting and oral fibromas.

The most severely affected system is the central nervous system resulting in cerebral manifestations in up to 95 percent of affected individuals.[2] Brain involvement includes cerebral hamartomas, subependymal nodules, and subependymal giant cell astrocytomas. The hamartomas, or tubers, are regions of abnormal cortical architecture with distinctive large neuronal cells[18] occurring at the gray-white junction. Varying in size from millimeters to several centimeters, tubers are rounded or wart-like protrusions of single or adjacent gyri, very firm to touch and pale in color. [8,13,19,20] Tubers are found primarily in the frontal-parietal cortex and cerebellum and rarely affect the brainstem and spinal cord. The number and size of the cortical tubers varies greatly between patients and correlates with the severity of neurologic manifestations. Goodman and colleagues[21]

noted statistically significant associations using the cortical tuber count detected on MRI scans as a biomarker to predict the severity of cerebral dysfunction in TSC patients.[12] Goodman's findings suggest that patients with moderate to severe cerebral dysfunction are more likely to have more than 7 MRI-detected tubers than mildly affected TSC patients. Subependymal nodules (SEN) are nodular lesions occurring in approximately 80 percent of TSC patients, are found mostly on the surfaces of the lateral ventricles, and are typically asymptomatic. SENS that continue to grow form subependymal giant cell astrocytomas (SEGAs).[22] SEGAs typically arise in the subventricular region of the brain, most commonly in the lateral ventricle, and can cause hydrocephalus and increased intracranial pressure.[23-25] SEGAs occur in 5 to 14 percent of TSC patients.[26,27]

The predominant neurologic sequelae of TSC are seizures, mental retardation, and behavioral disturbances.[12] Seizures are a common manifestation occurring in close to 80 percent of patients, often develop prior to one year of age, and are more common in males. Patients with generalized seizures and severe mental retardation correlate to a larger number of tubers in the brain.[17] Intellectual disability occurs in approximately 50 percent of the patients and likewise is seen more often in males. While most mentally retarded patients also suffer from seizures, many tuberous sclerosis patients have seizures but are not intellectually impaired.[12,28] Patients who develop seizures during their first five years of life seem to be the most likely to develop learning difficulties. Various behavioral problems occur in over 50 percent of TSC patients and include autism and attention-deficit with hyperactivity disorder. Other behavioral problems include strong anger or rage reaction, stubbornness, lethargy, and interrupted sleep patterns.

Retinal lesions can occur in up to 75 percent of TSC patients and include achromic patches, plaque-like hamartomas, and calcified astrocytomas. Most of the retinal lesions are of no clinical significance; however, there have been some reports of associated visual compromise.[28]

Cutaneous lesions occur in up to 95 percent of patients with TSC and include hypomelanotic macules (ash leaf spots), facial angiofibromas (adenoma sebaceum), ungual fibromas, and subcutaneous thickening (shagreen patches). Hypomelanotic patches occur at all ages and are seen in at least 90 percent of patients. The patches, as large as 5 cm in length, are most easily detected with an ultraviolet light (Wood's light). These lesions are frequently seen in the general population and are thus not pathognomonic of TSC. Facial angiofibromas, previously referred to as adenoma sebaceum, are papillary lesions that are pink in color and consist of blood vessels, fibrous tissue and sclerotic collagen (cutaneous hamartomas), typically in a butterfly distribution on the face, cheeks, nasal folds, and chin. The lesions usually do not become apparent until late childhood or adolescence.[9,12] A variation of the facial angiofibroma is the forehead fibrous plaque. This is a slightly elevated flesh-colored or yellowish plaque seen in slightly less than 20 percent of TSC individuals. Ungual fibromas appear during adolescents and adulthood and occur in approximately 25 percent of affected patients. These growths are fairly specific for TSC and are a major diagnostic feature. Shagreen patches (connective tissue nevus) are areas of thickened subcutaneous tissue manifested as raised yellowish-brown, pink, or green plaques or nevi found usually on the patient's lower back. These lesions are not specific for TSC but are considered a major diagnostic feature.

Renal involvement includes angiomyolipomas and multiple epithelial cysts (polycystic kidney disease). Angiomyolipomas are vascular tumors consisting of blood vessels, smooth muscle, and adipose tissue.[23] Angiomyolipomas can occur in up to 80 percent of patients, frequently manifest bilaterally, and can increase in size over time especially in females. Complications of these lesions include malignant transformation, pain, and severe hemorrhage. Renal cysts occur in 15 to 20 percent of TSC-affected individuals and result in renal failure in less than 5 percent of affected patients. Polycystic renal disease tends to occur in infants and young children, whereas angiomyolipomas, with or without cysts, are more common in adults.[14] In fact, in a longitudinal study conducted by Ewalt et al.,[29] half of the renal cysts observed on initial evaluation spontaneously disappeared on follow-up examination. This phenomenon has not been noted to occur with angiomyolipomas.

Bone involvement has been noted in 66 percent of patients affected with TSC.[30] Osseous findings reported include multiple areas of sclerosis, especially in the calvarium, spine, and pelvis, cyst-like lesions, and periosteal new bone formation in the hands and feet.[31]

Cardiac lesions consist of cardiac rhabdomyomas and are a frequent occurrence at or before birth. Fifty to 60 percent of individuals with TSC have evidence of cardiac disease, mostly rhabdomyomas.[9] These lesions usually have little clinical significance and tend to regress over the first few years of life.[2,32] Serious complications are uncommon and include arrhythmias, cardiomyopathy, and thromboembolic disease.

Pulmonary involvement is seen exclusively in women occuring in 1 to 6 percent of cases.[17] Lesions occur between 20 and 40 years of age and consist of either pulmonary cysts or lymphangioleiomyomatosis (LAM) leading to severe morbidity and mortality.

Many of the manifestations of tuberous sclerosis are age-dependent. Cardiac rhabdomyomas are frequently seen in infancy, which may give rise to arrhythmias and alterations in blood flow, but tend to regress with age with little morbidity. Facial angiofibromas, ungual fibromas, renal angiomyolipomas and SEGAs appear later in childhood or early adulthood.[33] Mortality from TSC is most commonly from development of an intracerebral giant cell astrocytoma or renal bleeding from an angiomyolipoma.

ORAL MANIFESTATIONS

The oral manifestations of TSC include pitting of the enamel, soft tissue fibromas, osseous fibromas of the jaws, and alveolar hyperostosis. There have been numerous reports describing the oral findings in patients of various age ranges, and about the nature of enamel pitting. Dental enamel pits have been found in pediatric and all adult patients with TSC, compared with 7 percent of controls.[34-37] There have been no reports, however, correlating the severity of enamel pitting, or oral fibromas, to the severity of the disease or to specific TSC mutations.

Enamel pitting as a feature of tuberous sclerosis was initially described by Hoff et al.[35] in 1975. The report included the clinical and scanning-electron microscope appearances of enamel defects in six patients with tuberous sclerosis. Clinically, an average of three pits were detected per tooth; the pits were randomly distributed, and

appeared without contralateral symmetry. Hoff described three types of defects: small pits (4 um in diameter), indentations (up to 60 um in diameter), and craterlike structures (up to 100 um in diameter). Electron microscope examination revealed depth of the enamel defects ranged from one-third of the enamel thickness to the base of the dentinoenamel junction. At the base of the pits, amorphous loose organic material and calculus were noted. No dentin abnormalities were noted. The pitting was assumed to be due to malfunctioning of one or more ameloblasts and a defective interaction between the odontoblasts and ameloblasts. Hoff concluded that the pitting was pathognomonic of TSC and that detection was important in the early diagnosis of the complex.

Sampson et al.[38] examined 30 patients ranging from age 10 to 45+ years old with tuberous sclerosis for macroscopic enamel pitting. They found 48 percent of permanent teeth showed pitting, while none of the six patients in the primary dentition had evidence of pitting. Enamel pits were found predominantly on the anterior teeth, and all but two pits affected the facial rather than the lingual surface. He concluded that a dental survey for enamel pitting may be useful in patients with permanent teeth, but is less helpful in the pre-school child.

Mlynarczyk[39] examined 50 patients with tuberous sclerosis and 250 control patients. He found the incidence of enamel pitting in the permanent dentition of patients with tuberous sclerosis was 100 percent, whereas in the adult dentition of the control group the incidence was 7 percent. The pitting in the control group was not as deep and definite as that of tuberous sclerosis patients. Among patients with tuberous sclerosis younger than 11 years of age, Mlynarczyk reported an incidence of 76 percent.

Another study by Lygidakis and Linderbaum[40] examined 36 families with TSC including 49 affected persons and 68 apparently unaffected first-degree relatives for pitted enamel hypoplasia. The study revealed enamel pitting in 71 percent of TSC patients and in 13 percent of the relatives.

There have been a couple of reports that have specifically described enamel pitting in deciduous teeth. Ho et al.[34] in 1995 published a report after examining enamel pitting in 13 TSC patients aged 2.5 to 18 years with varying degrees of TSC. A control group of 39 unrelated patients without TSC were also examined. A total of 77 percent of TSC patents revealed enamel pitting, compared with 13 percent of controls. The total number of enamel pits in each patient varied from 1 to 26 and increased with age. Ninety percent of the teeth with enamel pitting displayed one to two pits per tooth. The youngest patient with enamel pitting was 5 years old. All TSC patients older than 5 years and 1 month displayed pitting. In addition, Russel et al.[41] in 1996 examined shed deciduous teeth from 20 TSC patients for enamel pitting with a surface microscope. Enamel pitting was found in all 87 deciduous teeth, but in none of the 253 deciduous teeth from 142 controls constituting patients with cerebral palsy, phenylketonuria, and Down syndrome, as well as healthy persons. Enamel pits were always found on the facial surface of the central incisors, lateral incisors, and canines, but not in any specific location on the tooth. Ground sections examined microscopically revealed an undisturbed pattern of incremental lines (Retzium striae) surrounding the pits. Additionally, Russell reported the macro- and microscopic studies of the operculae from patients with TSC showed a very irregular hyperplastic inner surface of the dental follicle. He suggested that the hyperplastic changes of the inner enamel epithelium may be a cause of focal disturbances

of the amelogenesis. He concluded that the detection of enamel pits in deciduous as well as in permanent teeth is a valuable clinical diagnostic tool.

Enamel pitting is a form of hypoplasia and is not pathognomonic of tuberous sclerosis. Bhat reported enamel pitting as occurring twice as often than in the general population in patients suffering from cerebral palsy, mental retardation, and those with hearing defects.[42] Other conditions with enamel pitting include chemical ingestion during tooth formation (eg. fluoride, tetracycline), pseudohypoparathyroidism, amelogenesis imperfecta, vitamin D dependent rickets, epidermolysis bullosa, tricho-dento-osseous syndrome, and localized trauma during tooth development. Additionally, enamel pitting observed in individuals with or suspected of TSC must be distinguished from pitting associated with normal tooth morphology as well as certain types of smooth surface decay.

Other oral findings are of equal importance as diagnostic features of tuberous sclerosis. Oral lesions other than enamel pitting consist mainly of fibrous growths affecting the gingiva. They may also be found on the lips, the dorsum of the tongue, and the palate.[43,44] Although a prevalence of 11 percent has been reported for oral fibromas, the true frequency of these findings may be significantly greater; one study of 39 patients revealed oral fibromas in 56 percent of TSC patients.[43,45] Other oral abnormalities described include high palate, cleft lip and palate, macroglossia, and hyperostotic alveolar process.[43,46]

Lygidakis[43] in 1989 reported on oral fibromatosis after examining 36 families with TSC. The subjects consisted of 48 affected persons and 69 apparently unaffected parents and children along with 50 control subjects. Patients' ages ranged from 16

months to 62 years. Oral fibromatosis was observed in 56 percent of patients with TSC. None of the control subjects displayed oral fibromas. The fibromas were primarily found on the anterior gingiva in both jaws, were covered by normal mucosa and were of normal color. Severely affected patients had many large fibromas (up to 1 cm in diameter) whereas mildly affected patients had fewer numbers and of smaller size. Within the group of severely affected patients, the older patients appeared to have a larger number of fibromas. The youngest patient with oral fibromas was 11 years old. Oral fibromas have been reported to be histologically either fibromatous or papillomatous hyperplasias.[43,47,48]

Damm et al.[45] in 1999 reported on four patients with TSC with intraosseous fibrous lesions of the jaws. Radiographically the lesions were reported to be either a unilocular or multilocular radiolucency with well-defined or ill-defined borders. Histopathologically, Damm et al. described the lesions having numerous basophilic and slightly irregular spindle-shaped nuclei within dense fibrous connective tissue that revealed a swirling pattern. Similar lesions can occur in extragnathic bone. The authors concluded that the intraosseous fibrous proliferations represent a manifestation of tuberous sclerosis rather than a coincidental finding. Additionally, the authors suggest that radiographic studies of the jaws should be a part of the diagnostic evaluation of any patient suspected of having tuberous sclerosis.

GENETICS

TSC is characterized by genetic heterogeneity.[31,49] Linkage studies have identified loci for TSC on chromosomes 9q34 (TSC1) and 16p13.3 (TSC2) and suggest that they account for approximately equal numbers of families.[50,51] There is no evidence for

additional TSC loci. The TSC1 gene was identified in 1997 and spans 40 kb with 23 exons and messenger RNA of 8600 nucleotides (See Appendix I). The gene codes for the TSC1 protein, hamartin, contains 1164 amino acids with a predicated mass of 130 kDa .[51] The function of hamartin is unknown. The TSC2 gene was identified in 1993 and is encoded by 41 exons, spanning approximately 50 kb of genomic DNA 11[52] (see Appendix I). The 5474 nucleotide mRNA encodes the protein tuberin, which has a region of sequence homology to the GTPase activating protein rap1 GAP.[53] Tuberin functions as a rap1 regulator possessing negative growth regulatory properties. The TSC2 gene is adjacent, in a tail-to-tail fashion, to the PKD1 gene, which is the major gene involved in autosomal dominant polycystic kidney disease.[54] Loss of heterozygosity (LOH) for DNA markers for both the TSC1 and TSC2 regions suggest that both tuberin and hamartin act as tumor suppressors.[23] LOH in hamartoma suggest that a second somatic mutation may be required to produce the TSC phenotype at a cellular level and is consistent with the genes acting as tumor suppressors.[54] The two-hit hypothesis, as proposed by Knudsen, states that a germline alteration in the tumor suppressor gene, inherited from an affected parent, is complemented by a second somatic alteration in the allele inherited from the unaffected parent.[55] Development of tumors arises from two independent mutational events. The inactivation of both copies of the gene causes a loss of growth control and the cell and its progeny proliferate as a hamartoma.[56] The exact function and relation of the gene products is not completely understood. Tuberin might have important functions relevant to neuronal differentiation and development, and it is speculated that tuberin and hamartin may function together participating in common pathways.[4,57]

Approximately two-thirds of TSC cases are sporadic, occurring in the absence of a family history of the disorder.[10,18,49] These cases are thought to arise by mutation. Jones reported out of 150 unrelated TSC cases studied (130 sporadic, 19 familial), 120 had detectable disease-causing mutations and 18 percent were at the TSC1 locus, and 82 percent at the TSC2 locus.[4] Jones also found that significantly more of the sporadic cases had TSC2 than TSC1 mutations. In familial cases, there is equal distribution between TSC1 and TSC2 cases. It has been proposed that overall disease severity is greater in TSC2 than in TSC1, and this may explain the disproportionate numbers of TSC2 mutations among sporadic cases. In fact, intellectual disability was found to be significantly more frequent among sporadic cases with TSC2 mutation than among those with TSC1 mutations.[4] Jones reported that phenotypic expression in familial TSC tends to be more mild because severely affected individuals rarely reproduce.[3]

Mutations result from either deletions, insertions, or rearrangement of the base pair, or nucleotide, sequence in DNA. It is the sequence of base pairs in DNA that determines the amino acid sequence of the encoded protein. Mutations can be defined as follows[58,59]:

Missense mutations: An alteration in a coding sequence of DNA that results in an amino acid replacement in the polypeptide.

Nonsense mutations: A mutation that changes a codon specifying an amino acid into a stop codon, resulting in premature polypeptide chain termination.

Frameshift mutation: A mutation caused by the insertion or deletion of one or more nucleotide pairs in a gene, resulting in a shift in the reading frame of all codons following the mutational site.

Large deletions: A mutation that occurs as a result of the loss of an entire portion of the DNA sequence.

Large insertions: Insertion of segments of extra bases often from another part of a chromosome.

Rearrangements: Mutations resulting from segments of DNA sequence exchanging position with each other.

Mutations resulting in a stop codon (mRNA codons UAG, AUU, or UGA) lead to the termination of polypeptide synthesis and are referred to as truncating mutations. Nontruncating mutations result from a change in amino acid sequence and lead to an alteration in the biological properties of the produced protein.

A wide variety of underlying genetic alterations have been identified in TSC including large and small deletions, insertions, nonsense mutations, and missense mutations. Several studies have shown the great majority of germ-line mutations at the TSC1 locus to be small truncating lesions.[3,5,51,60,61] Jones demonstrated that at the TSC2 locus, missense changes, large rearrangements including whole-gene deletions, nonsense mutations, and small insertions and deletions are represented at similar frequencies.[4]

Au determined that TSC2 mutations can be divided into two groups: those predicted to lead to premature termination of tuberin (frameshift insertions/deletions and nonsense mutations) and those that do not lead to premature termination of tuberin (missense mutations and in-frame deletions).[52] He concluded that the type of germ-line mutation does not influence clinical phenotype. Thus far, 148 unique mutations in TSC1 and 251 unique mutations in TSC2 (see table) have been reported (http://zk.bwh.harvard.edu/projects/tsc_database/index.html).[62] Fifteen or more of these have been seen

recurrently, but none accounts for more than 5 percent of all patients.[4,5,52,60-67] Eighty-eight percent of mutations are small changes including small deletions or insertions (38 percent), and nonsense, splice site, or missense point mutations (50 percent). Large genomic deletions and rearrangements account for 12 percent of identified mutations and are seen primarily in TSC2.[62]

Because the structure of the TSC2 gene is more complex that the TSC1 gene, and its mutational spectrum more diverse, TSC2 mutation detection is more limited. Additionally, a substantial fraction of sporadic cases could involve mosaicism. Mosaicism is the phenomenon in which a fraction of, rather than all, germ-line somatic cells contain a mutation or chromosomal abnormality.[18] Through mosaicism, it is possible for mutations to be present at low frequency, despite the presence of severe disease. This may account for the limitations of molecular diagnostic methods, and why relatively few mutations have been identified thus far in patients with TSC.[3,18,52,60,66]

With the exception of a contiguous gene deletion syndrome involving TSC2 and PKD1 at 16p13.1, and less mental handicap among TSC1 mutations as reported by Jones,[3] significant differences between TSC1 and TSC2 phenotypes have not been demonstrated.[3,50] Additionally, to date, no definitive genotype-phenotype correlation has been established. With the identification of TSC1 and TSC2 genes, along with increasing sophistication of mutation detection, it should be possible to more accurately analyze phenotype differences in sporadic versus familial cases as well as evaluate for genotype correlation.

METHODS AND MATERIALS

This study is part of a larger IRB-approved investigation (CHMC 98-1-4) at Children's Hospital Medical Center, Cincinnati, Ohio, by the Department of Neurology, "Genotype/Phenotype Analysis in Individuals with Tuberous Sclerosis and Their Family Members." The goal of this study is to determine if any genotype/phenotype correlations exist in patients with TSC1 or TSC2. Written informed consent was obtained for each clinical examination and DNA analysis per the IRB protocol. The informed consent included permission for a general physical examination and included an oral examination. Subjects were recruited from the Tuberous Sclerosis Center at Children's Hospital Medical Center, Cincinnati, Ohio, and all had a clinically definite diagnosis of tuberous sclerosis. There were no controls, and subjects were not randomized.

ORAL EXAMINATIONS

All oral examinations were completed in the Tuberous Sclerosis Center at Children's Hospital Medical Center. A total of 104 exams were completed: 99 exams were completed by the author, and 5 exams were completed by a pediatric dental resident. Study subjects brushed their own teeth or had their teeth brushed to remove as much plaque as possible prior to staining the facial surfaces with Trace® disclosing solution (Red dye #28) using a 6-inch cotton swab. The teeth were examined using an explorer and a lighted mirror (DenLite® by WelchAllyn). Upon completion of the exam, patients again had their teeth brushed to remove the dye stain. As a standardized protocol

in data collection, an examination form was used (See Appendix II) to tabulate the following:

1. The presence or absence of primary and permanent teeth.

2. The total number and location of pits affecting the facial surfaces of the maxillary and mandibular incisors, canines, and first and second molars in the primary dentition were recorded. The total number and location of pits affecting the maxillary and mandibular incisors, canines, premolars, and first molars in the permanent dentition were recorded.

3. Size of pits – Pits were categorized into one of two pit sizes and charted as follows:

Pinpoint Size: The width of the tip of a dental explorer or smaller.

Crater Size: The width greater than the tip of a dental explorer.

4. Gingival fibromas – the total number and location of fibrous growths were recorded.

TSC PHENOTYPE VARIABLES

Patients participating in the study all had extensive testing, which included any or all of the following: MRI scans of the brain, echocardiography, renal ultrasound, an opthalmic examination, a neuropsychologic assessment, blood samples for genotype analysis, and an oral examination. As part of the larger IRB-approved study, one blood sample was obtained from each individual. Five to 10 ml of whole blood was obtained from individuals 1-10 years of age. Ten to 20 ml of blood was obtained from patients greater than 10 years of age. At no time did the total amount of blood removed from

each individual exceed 5 percent of their estimated total blood volume. All blood samples were obtained by the Test Referral Center at Children's Hospital Medical Center. Universal blood and body fluid precautions were followed.

Confidentiality of a patient's identification was maintained. Genotype test results were not included in that patient's medical record; results were not divulged to any third party and were known only by investigators participating in the study. If individual subjects or family members desired the result of their genotype analysis, this was verbally communicated to them by the Division of Human Genetics at Children's Hospital Medical Center. Results of the genotype analysis were correlated with the phenotype of particular individuals only for the purpose of statistical analysis and to ascertain whether certain oral manifestations are associated with certain genetic mutations.

METHOD OF DNA EXTRACTION

DNA was extracted from leukocytes using a technique of isolation by differential affinity at Children's Hospital Medical Center. QIAamp DNA Blood Kits (QIAGEN®) for large-scale genomic and viral DNA purification was used to isolate pure DNA ready for amplification. Using this system, separation of leukocytes from whole blood was not necessary. The procedure consisted of the following steps:

1. Five-10 ml of blood was added to a 50 µl centrifuge tube.
2. Five hundred µl of QIAGEN Protease stock solution and 12 ml of Buffer AL was added and mixed thoroughly by vortexing.
3. Mixture was incubated at 70 °C for 10 minutes.
4. Ten µl of ethanol was added to the sample and mixed by vortexing.

5. Half of the solution was applied onto the QIAamp Maxi column and placed in a 50-ml centrifugation tube and centrifuged at 3000 rpm for 3 minutes.

6. The QIAamp Maxi column was removed, the filtrate discarded, and the Maxi column was placed back into the 50-ml centrifugation tube. The remaining half of the solution was applied onto the QIA Maxi column, placed in the centrifugation tube and centrifuged at 3000 rpm for 3 minutes.

7. The QIA Maxi column was removed, the filtrate discarded, and the column placed back into the centrifugation tube.

8. Five ml of Buffer AW1 was added to the Maxi column and was centrifuged at 5000 rpm for 1 minute.

9. Five ml of Buffer AW2 was added to the Maxi column and centrifuged at 5000 rpm for 15 minutes.

10. The Maxi column was removed and placed in a clean 50 ml centrifugation tube, and 1 ml of Buffer Acetate EDTA was added onto the membrane of the column, incubated at room temperature for 5 minutes, and centrifuged at 5000 rpm for 5 minutes.

11. A 1:100 dilution of the sample was then placed in a Beckman Spectrophotometer to evaluate the yield.

METHOD OF DNA MUTATION ANALYSIS

Once an adequate yield was confirmed, samples were sent to Brigham and Women's Hospital in Boston, Mass. for analysis for mutations of either of the tuberous sclerosis genes by David Kwiatkowski, M.D., Ph.D. These results were then communicated to the investigators at CHMC and made available for the dental study.

Denaturing high performance liquid chromatography (DHPLC) was used for exon by exon mutation detection of point mutations and small insertions/deletions in TSC1 and TSC2 genes. This was followed by a long-range PCR assay for identification of large deletions in TSC2. DHPLC is a recently developed method for DNA variation detection that uses reversed phase ion pair liquid chromatography to detect DNA heteroduplexes.[62,68-70] Its advantages over other methods of DNA analysis include superiority in detection of DNA sequence variation as well as its semi-automation. Up to 96-well tray samples can be loaded on the apparatus at one time and subject to analysis over several hours. The process involved the following steps:

1. The DNA sample was diluted 1:20 and gel electrophoresis was run on a 0.7 percent agarose gel to verify DNA presence.

2. Samples were then subjected to a PCR reaction for amplification of exons using a PTC-100 thermal cycler with primers and buffers. Primer sequences for PCR amplification of TSC2 exons can be found at http://zk.bwh.harvard.edu/projects/tsc/. The DNA was denatured to single strands by adding EDTA to the sample and heating to 95 °C for 5 minutes. Reannealing to form heteroduplexes products occurred after 1 hour at 65 °C.

3. Heteroduplexes were analyzed using the DHPLC WAVE ™ DNA Fragment Analysis System (Transgenomic, Inc., San Jose, CA).

4. Chromatograms were established for each DNA fragment amplicon and were compared with controls to detect samples with probable mutations.

5. Sequence variation was identified by loading the samples onto an ABI gel for automated sequencing using an ABI 377 machine (Perkin-Elmer). Sequencing was bi-

directional along with controls to minimize errors. The data from the ABI chromatogram was transferred to a computer with software capable of detecting mutations.

6. Samples were screened for large TSC2 deletions using long-range PCR methods that use a series of PCR primers and agarose gel electrophoresis. Long PCR-based assay utilizes primers that amplify overlapping segments of the TSC2 gene to increase the probability of finding all deletions.

TABULATION OF DATA

Results of the neuro-imaging, neuropsychological, renal imaging, echocardiogram, TSC mutations, and oral findings were entered into a database created in Microsoft Access® for tabulation purposes (See Tables I, II and III). Variables for phenotype correlations included a history of polycystic kidney disease (PKD), cardiac rhabdomyomas, arrhythmias, facial angiofibromas, renal angiomyolipomas, and retinal disturbances. Correlations were also made to the total number of cortical tubers, the degree of cognitive impairment, seizure severity, and the total number of antiepileptic drugs required to control seizures (AEDs).

MUTATION GROUPING

Due to the limited number of TSC1 patients, mutation analysis was limited to TSC2 patients. Mutations were grouped as either resulting in truncating mutations leading to premature termination of the protein or as nontruncating mutations resulting in an altered protein. Truncating mutations included small deletions or insertions, nonsense, splice site, frameshift mutations. Large genomic deletions that result in no protein

product were also included in this category, because the effect is essentially the same as truncation. The nontruncating category included missense mutations and in-frame deletions.

STATISTICAL METHODS

To compare means of oral findings and angiomyolipomas (AML) polycystic kidney disease (PKD), cardiac rhabdomyomas, cardiac arrhythmias, facial angiofibromas, retinal lesions, seizure activity, familial vs sporadic disease, and mutation categories, the two sample t-test was used. The one-factor analysis of variance (ANOVA) was employed to evaluate oral findings against seizure severity and the degree of cognitive impairment where the number of groups was more than two. If the results from ANOVA were statistically significant, the Bonferroni adjustment factor was applied to determine which pairwise groups had different means. The chi-square was used to test for differences in proportions in examining the appearance of oral fibromas with AML, PKD, cardiac rhabdomyomas, cardiac arrhythmias, facial angiofibromas and retinal fibromas. Pearson's correlation coefficient was computed for pairs of quantitative phenotype variables. Spearman's rho was used to examine the relation between oral findings and categories of phenotype variables.

SAS Version 7.0 was used for T-tests, ANOVA, and chi-square analyses, while SPSS version 9.0 was used for correlation analyses.

RESULTS

ORAL FINDINGS

Enamel Pitting

A total of 104 patients with a clinical diagnosis of tuberous sclerosis were examined for enamel pitting and gingival fibromas over a two-year period from 5/13/98 to 6/7/00. A total of 46 males (44 percent) and 58 females (56 percent) were included in the study. Of the total, 31 patients (30 percent) were in the primary dentition, 19 patients (18 percent) in the mixed dentition and 54 patients (52 percent) in the permanent dentition. The age range of patients examined was from 1 year to 51 years of age. These data are shown in Tables I part I-V and summarized in Figure 4.

Collectively, the study subjects had a total of 2223 teeth examined. Of the 1487 permanent teeth examined, 604 (41 percent) had enamel pitting with 83 percent of the pitting classified as pinpoint-sized pits, and 17 percent as crater-sized pits. Seven hundred and thirty-six primary teeth were examined of which 53 (6 percent) displayed pitting, with 91 percent of the pitting classified as pinpoint, and 9 percent as craters sized pits. These data are summarized in Figures 6 and 7.

Twenty-nine percent of the patients in the primary dentition group had enamel pitting with an average of 2.2 pits per affected patient with a maximum of 4 pits per individual patient. Ninety percent of those in the mixed dentition group had enamel pitting with an average of 10.8 pits per affected patient and a maximum of 41 pits per individual patient. One hundred percent of patients in the permanent dentition group had

enamel pitting with an average of 16.4 pits per individual and a maximum of 68 pits per individual patient. These data are summarized in Figures 5, 7, and 14

Over 60 percent of enamel pitting occurred in the maxillary arch for both primary and permanent teeth with the majority of enamel pitting occuring anteriorly for both sets of teeth (see Figures 8 and 9). The maxillary canine was the most common primary tooth affected, with a pitting occurrence of 24 percent (see Figure 11) and also displayed the greatest number of enamel pits of all primary teeth with an average of 0.26 pits (see Figure 12). The average number of enamel pits for all primary teeth was 0.07. The maxillary central incisor was the most common permanent tooth affected, with a pitting occurrence of 78 percent (see Figure 10) and displayed the greatest number of pits of all permanent teeth with an average of 1.42 pits. (see Figure 13). The average number of enamel pits for all permanent teeth was 0.70 pits.

Gingival Fibromas

Gingival fibromas were seen in 47 percent of patients between 6 and 13 years of age, and in 70 percent of patients in the permanent dentition (see Figure 15). Only one patient under the age of six years old had a gingival fibroma. It was located anteriorly in the maxillary arch. The majority of gingival fibromas occurred in the maxillary arch (62 percent) and occurred anteriorly positioned (72 percent for patients in the mixed dentition and 77 percent for patients in the permanent dentition). These data are shown in Table X, Parts I-V and summarized in Figures 16 and 17.

The fibromas were primarily fibrous nodules covered with mucosa of normal color and located more often on the interdental papilla of the facial gingiva (see Figure 3). They varied from a few millimeters to 1 cm in size and all were asymptomatic.

The average number of gingival fibromas per affected patient was 6.4 for patients in the mixed dentition group, and 4.8 for patients in the permanent dentition group. A two-sample test failed to show a significant difference between the two means.

COMPARING ENAMEL PITTING TO PHENOTYPE VARIABLES

Phenotype Variables With a Lack of Significant Findings

Using T-tests and Spearman's rho correlation analyses, there were no significant findings between the degree or severity of enamel pitting in the primary or permanent teeth with AML, PKD, cardiac rhabdomyomas and hypertension and retinal lesions. These data are shown in Tables II, V, VI, VII, X and XV-Parts I and II and summarized in Table XVIII-Parts I, II and III.

T-tests did not show significant differences in the degree or severity of enamel pitting in either the primary or permanent teeth in patients with and without seizures. Likewise, the ANOVA for seizure severity failed to show significant differences with enamel pitting. Additionally, there were no correlations between the degree or severity of enamel pitting in either dentition and the total number of anti-epileptic drugs (AEDs) taken to control seizures. These data are shown in Tables XI, XV-Part I and XVI and summarized in Table XVIII-Part I.

The level of cognitive impairment was described as unaffected, mild, moderate or severe. Analyses of variance failed to show any significant differences between the level of impairment and degree or severity of enamel pitting in the primary or permanent teeth. These data are shown in Table XVI and summarized in Table XVIII-Part I.

Cardiac Arrhythmias and Facial Angiofibromas

There was a positive correlation between cardiac arrhythmias and the average number of enamel pits in primary teeth ($r = 0.408$; $p = 0.014$; $n = 36$) but not in permanent teeth; however, the t-test analyses between the two groups failed to show a significant difference. There was no correlation between the severity of pitting with cardiac arrhythmias. These data are shown in Tables II, VIII and XV-Part I and summarized in Table XVIII-Part II.

There was a positive correlation between the average number of pits in the primary ($r = 0.527$; $p = 0.002$; $n = 32$) as well as permanent ($r = 0.378$; $p = 0.011$; $n = 44$) teeth with the presence of facial angiofibromas. The t-test for both primary ($t = - 2.76$; $p = 0.009$) and permanent ($t = -2.62$; $p = 0.012$) teeth revealed a significant difference between the means. There was no correlation between severity of pitting with facial angiofibromas. These data are shown in Table IX and XV-Part II and summarized in Table XVIII-Part III.

Total Number of Cortical Tubers and IQ Estimation

IQ estimates were completed by a neuropsychologist and were based on actual test results (i.e. Bayley Scales of Infant Development, Wechsler Intelligence Scale for

Children, 3rd edition). Otherwise, IQ estimates were based on school and job performance and clinical judgments of disease severity. There was a negative correlation between the average number of enamel pitting in the primary teeth and estimation of IQ ($r = -0.405$; $p = 0.05$; $n = 24$).

Tuber count was determined by a radiologist from MRI imaging. Given the large number of tubers in most of the patients, a maximum of 10 tubers were counted in each of four examined areas. Patients with more than 10 tubers were assigned a count of 11 for the purposes of quantitative analysis. The maximum number of tubers in the scoring system was 44. There was a negative correlation between the percent of crater-sized pits in the permanent teeth and the total number of cortical tubers ($r = -0.587$; $p = 0.035$; $n = 13$). There was a positive correlation, however, between the percent of pinpoint-sized pits in permanent teeth and total number of cortical tubers ($r = 0.587$; $p = 0.035$; $n = 13$). These data are shown in Table XV (Part I) and summarized in Table XVIII (Part I).

COMPARING ENAMEL PITTING WITH TSC GENETICS

Familial vs. Sporadic Disease

There were no significant associations between the number and degree or severity of enamel pitting in the primary or permanent teeth with familial cases compared with sporadic cases of tuberous sclerosis. These data are shown in Tables III, XII and XV (Part III), and summarized in Table XIX.

Truncating vs. Nontruncating Mutations

There were no significant associations between the degree and severity of enamel pitting in the primary and permanent teeth with TSC2 mutations leading to truncated as compared with nontruncated protein products. These data are shown in Tables III, XIII and XV (Part III), and are summarized in Table XIX.

COMPARING GINGIVAL FIBROMAS WITH PHENOTYPE VARIABLES

Phenotype Variables Lacking Significant Findings

Using statistical tests consisting of t-tests, ANOVA, Pearson's correlation, Spearman's rho, and chi-square used separately or in combination, there were no significant findings between the number of gingival fibromas either anteriorly or posteriorly positioned with these phenotype variables. The data are shown in Tables V, VI, VII, X, XI, XII, XIII, XIV, XV (Parts I and II), XVI, and are summarized in Table XVIII (Parts I, II, and III).

Retinal Lesions

There was positive correlation between the total number of gingival fibromas and the appearance of retinal lesions ($r = 0.372$; $p = 0.020$; $n = 39$). The frequency of patients with gingival fibromas increased proportionally with retinal lesions [X^2 (1) = 4.96; $p = 0.0258$]. T-tests failed to show a difference between patients with and without gingival fibromas and retinal lesions. These data are shown in tables X, XIV, XV (Part II) and are summarized in Table XVIII (Part III).

Cardiac Arrhythmias

There were significant differences between the total number of gingival fibromas ($t = 2.65$; $p = 0.01$), both anteriorly ($t = 2.31$; $p = 0.02$) and posteriorly ($t = 2.18$; $p = 0.03$) positioned with the occurrence of cardiac arrhythmias. The Chi-square test did not show significance and correlation analyses failed to demonstrate a correlation. These data are shown in Tables VIII, XIV and XV (Part I) and are summarized in Table XVIII (Part II).

Facial Angiofibromas

There was a positive correlation between the total number of gingival fibromas and the occurrence of facial angiofibromas ($r = 0.514$; $p < 0.0001$; $n = 64$). There was a significant difference in the average number of gingival fibromas in patients with facial angiofibromas ($t = -3.32$; $p = 0.0015$) compared to those without, and the frequency of gingival fibromas increased in proportion with facial angiofibromas [X^2 (1) = 18.95; $p < 0.0001$]. These data are shown in Tables IX, XIV, and XV (Part II) and are summarized in Table XVIII (Part III).

COMPARING GINGIVAL FIBROMAS WITH TSC GENETICS

Familial vs. Sporadic Disease

There were no significant associations between the total number of gingival fibromas, either anteriorly or posteriorly positioned in patients with familial or sporadic

TSC. These data are shown in Tables XII and XV (Part III) and are summarized in Table XIX.

Truncating vs. Nontruncating Mutations

There were no significant associations between the total number of gingival fibromas, either anteriorly or posteriorly positioned, and TSC2 mutations leading to truncated or nontruncated protein products. These data are shown in Tables XIII and XV (Part III) and summarized in Table XIX.

FIGURES AND TABLES

FIGURE 1. Facial angiofibromas characteristic of Tuberous Sclerosis Complex

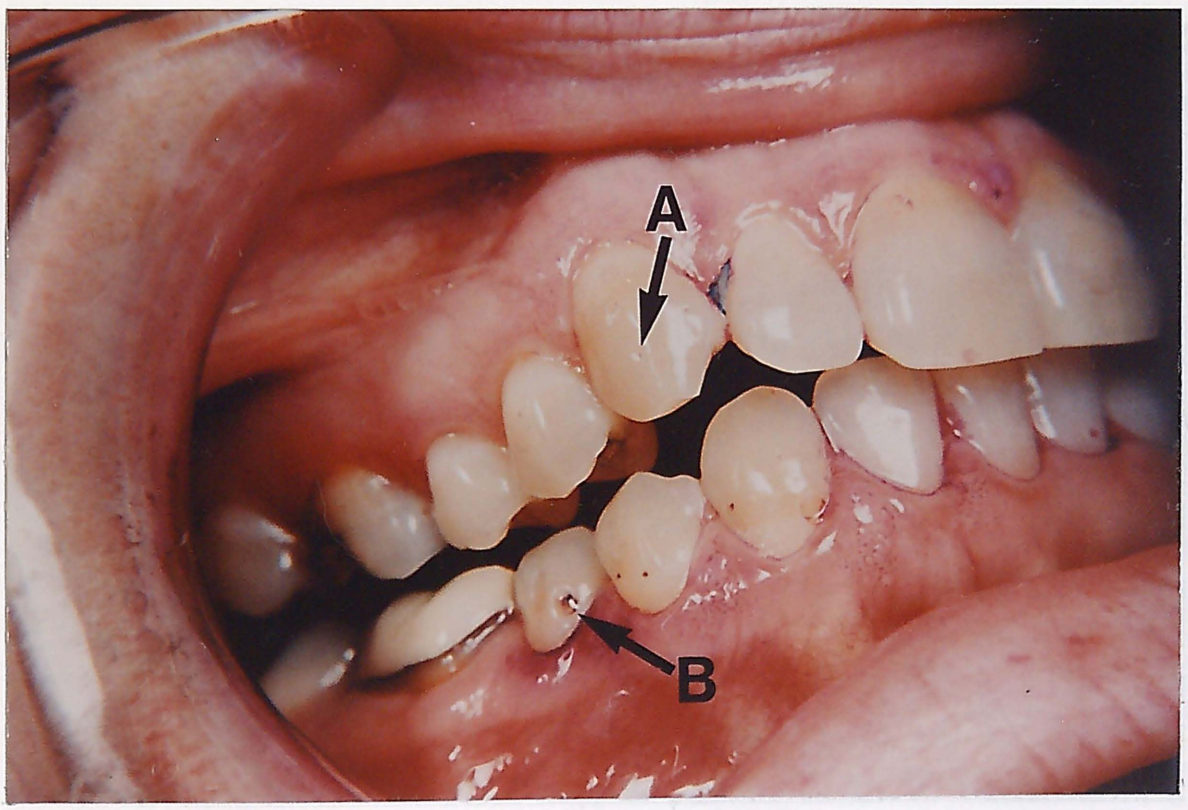


FIGURE 2. Enamel pitting. Pinpoint size enamel pitting (A) and crater size enamel pitting (B) characteristic of tuberous sclerosis complex. Red dye has become entrapped in the pits enhancing their recognition.

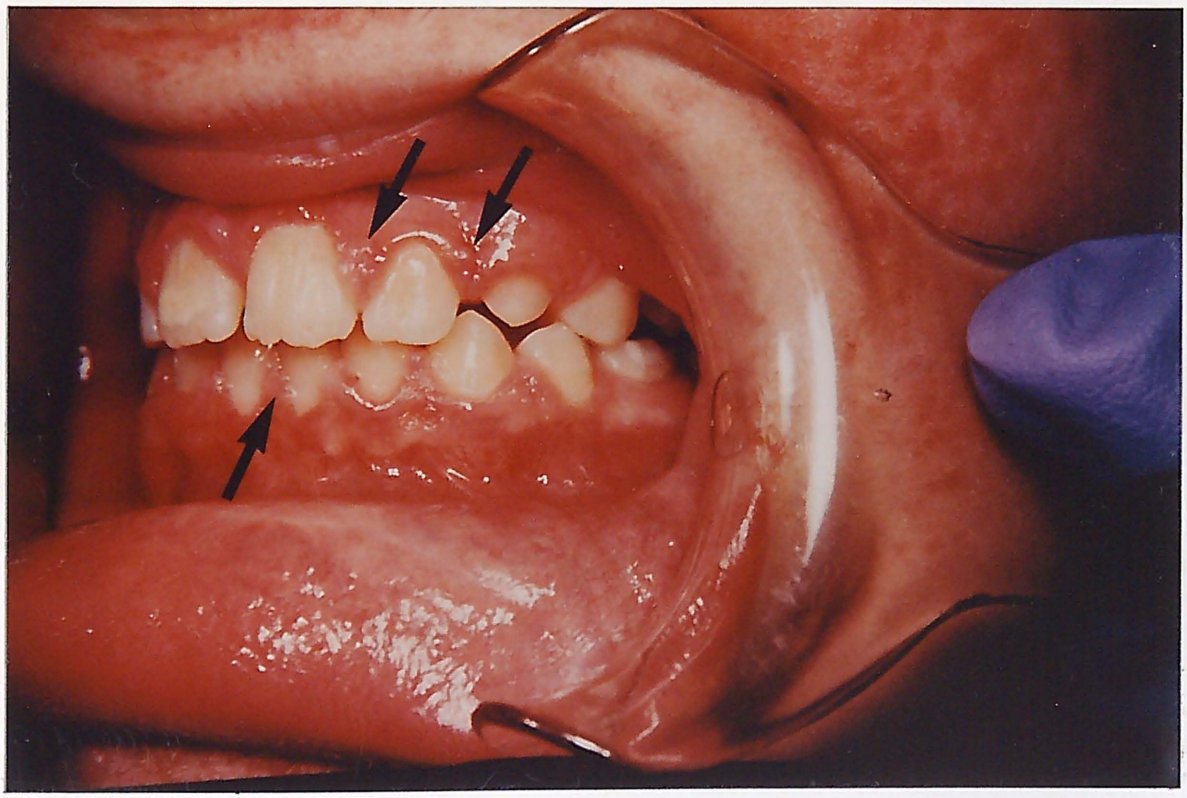
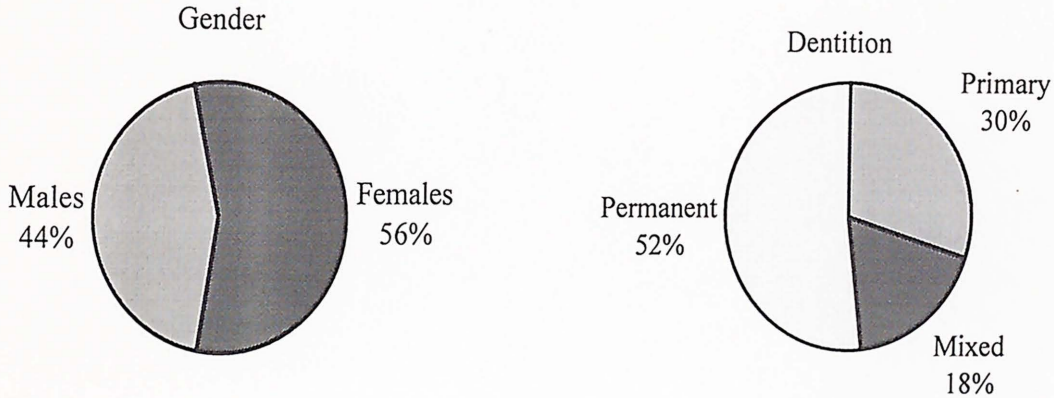
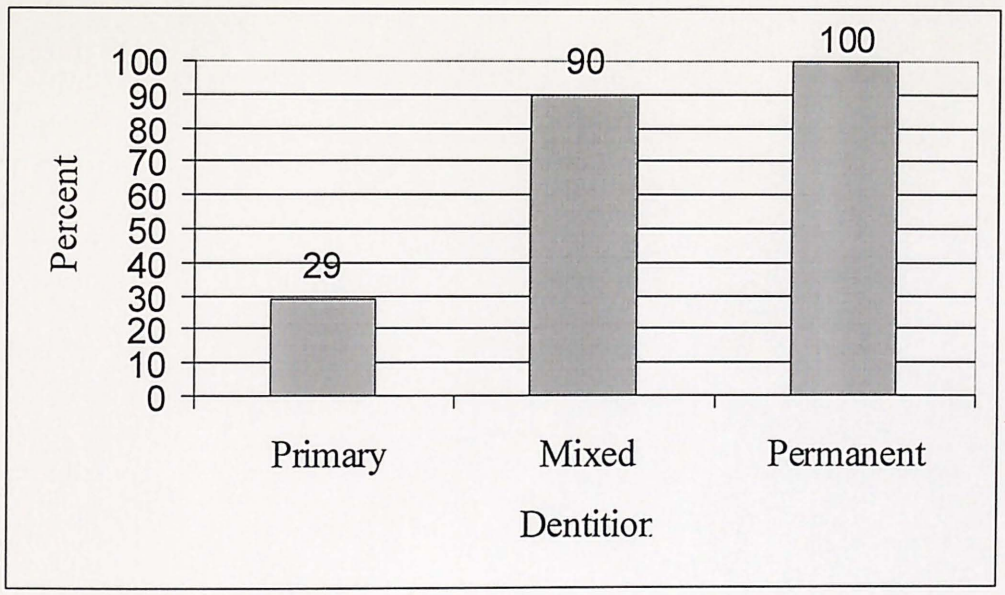


FIGURE 3. A gingival fibroma in a patient with Tuberous Sclerosis Complex



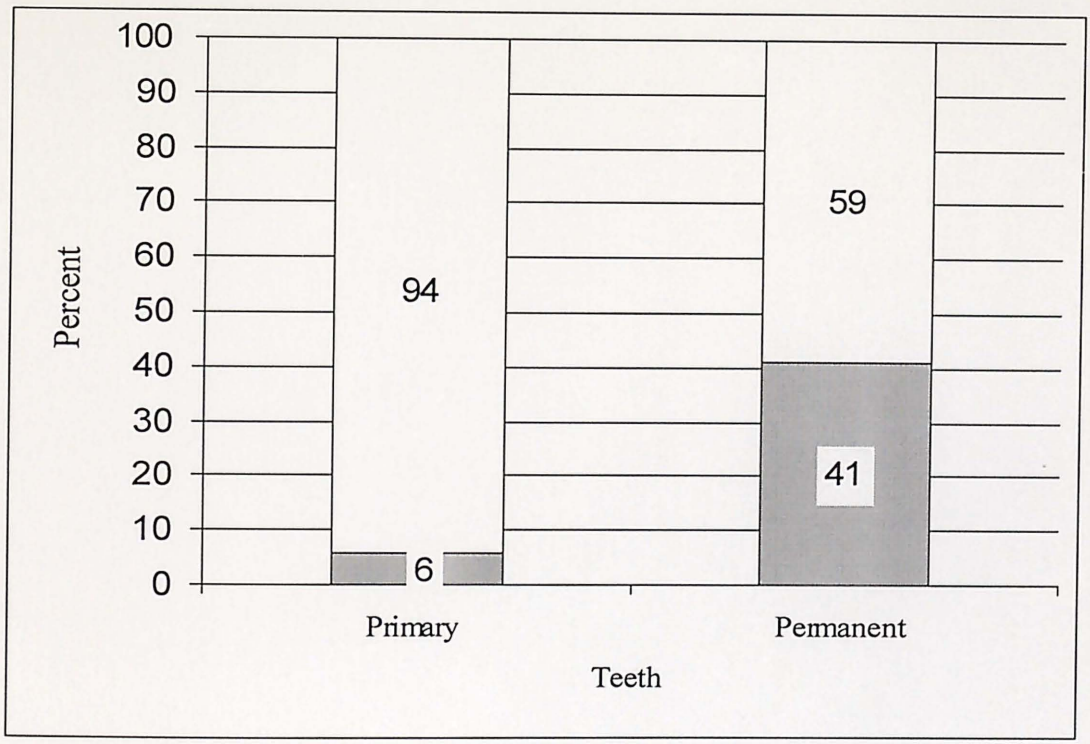
Dentition	Age Range	Males	Females	Total
Primary	1-6 years	16	15	31 (30%)
Mixed	6-13 years	14	5	19 (18%)
Permanent	11-51 years	16	38	54 (52%)
Total	1-51 years	46 (44%)	58 (56%)	104

FIGURE 4. Total number of patients examined with a clinical diagnosis of Tuberous Sclerosis Complex (TSC) by gender, age and dentition.



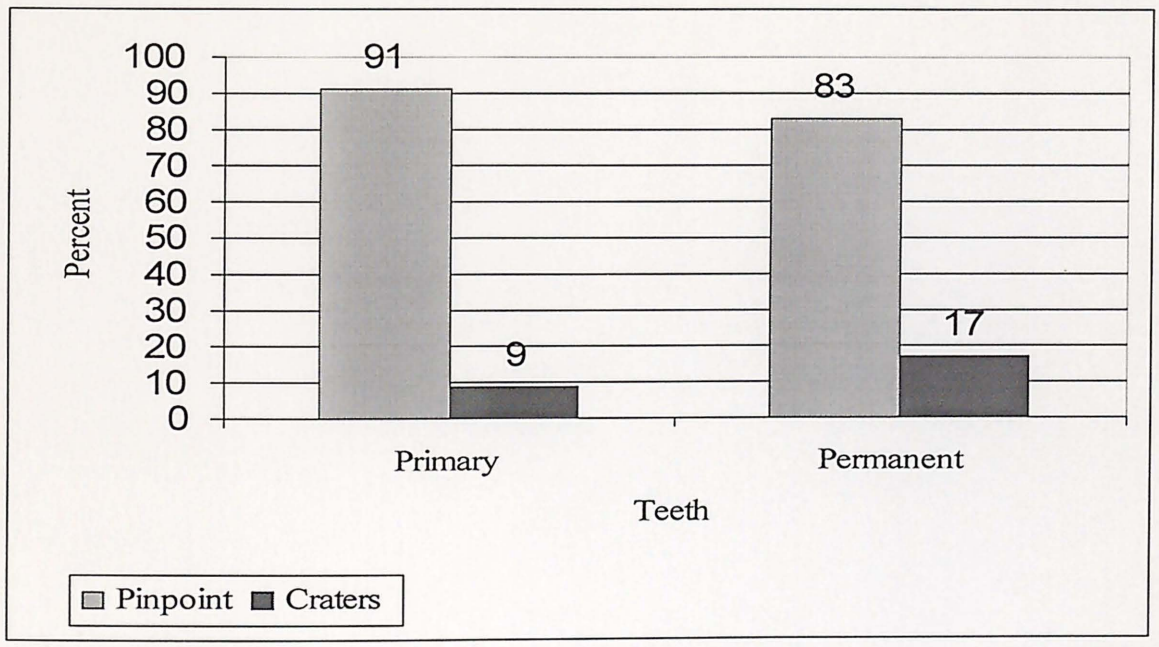
	Primary	Mixed	Permanent
Number of Patients	31	19	54
Patients with Pits	9 (29%)	17 (90%)	54 (100%)

FIGURE 5. Occurrence of enamel pitting in patients in the primary, mixed and permanent dentitions.



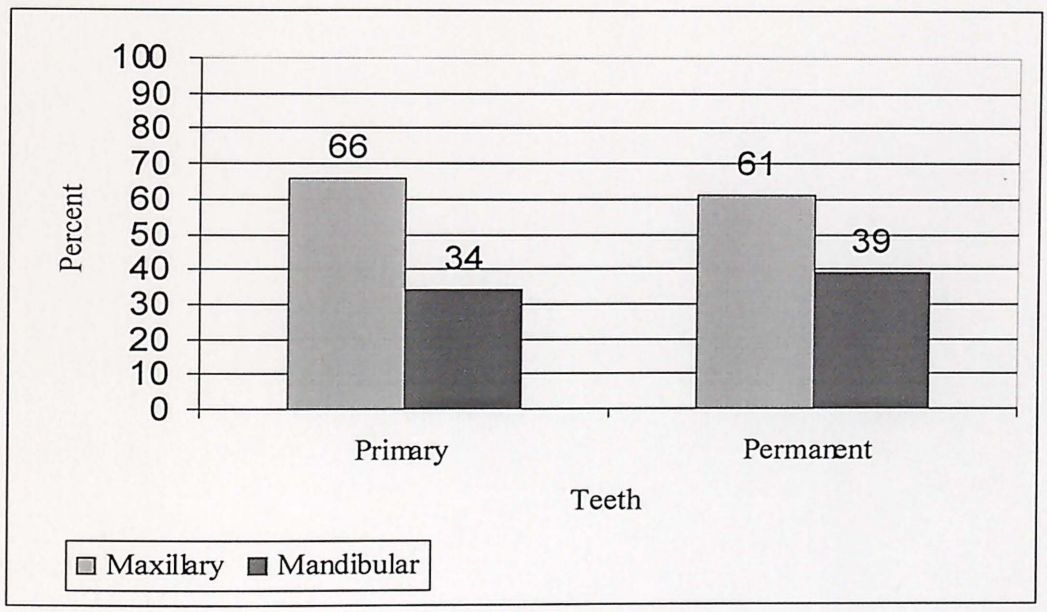
	Primary Dentition	Permanent Dentition
Teeth Examined	736	1487
Teeth With Pits	53 (6%)	604 (41%)
Teeth Without Pits	688 (94%)	883 (59%)

FIGURE 6. Total number of primary and permanent teeth examined affected by enamel pitting.



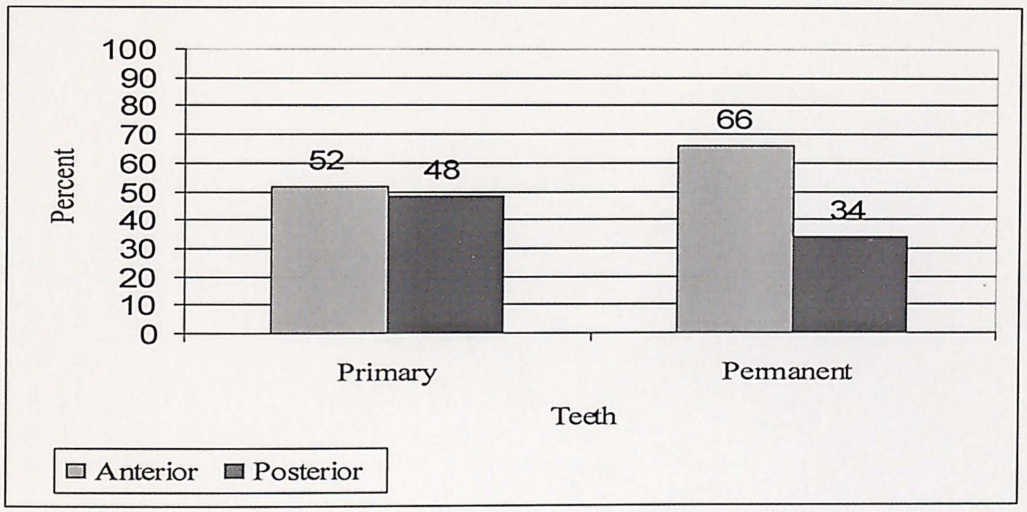
	Primary Teeth	Permanent Teeth
Number of Pits	58	1029
Pinpoint Pits	53 (91%)	853 (83%)
Crater Pits	5 (9%)	176 (17%)

FIGURE 7. Distribution of pinpoint vs. crater size enamel pitting affecting primary and permanent teeth examined.



	Primary Teeth	Permanent Teeth
Number of Pits	58	1029
Maxillary # of Pits	38 (66%)	625 (61%)
Mandibular # of Pits	20 (34%)	404 (39%)

FIGURE 8. Occurrence of enamel pitting affecting maxillary and mandibular arches in primary and permanent teeth examined.



	Primary Teeth	Permanent Teeth
Anterior		
Maxillary	26	453
Mandibular	4	225
Total Pits	30 (52%)	678 (66%)
Posterior		
Maxillary	12	172
Mandibular	16	179
Total Pits	28 (48%)	351 (34%)

FIGURE 9. Occurrence of enamel pitting affecting anterior and posterior arch positions in primary and permanent teeth examined.

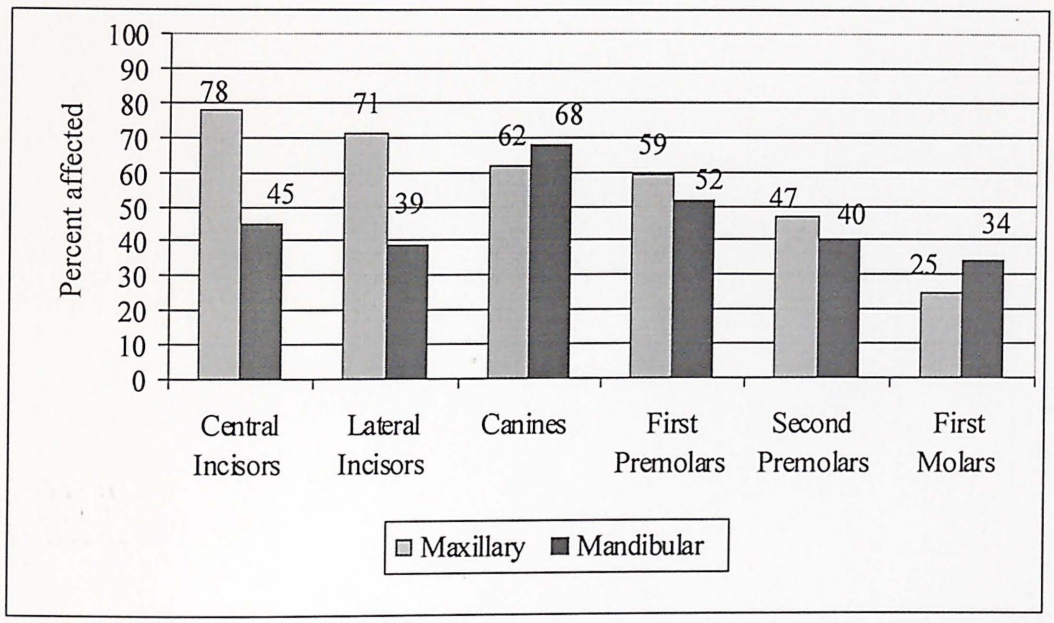


FIGURE 10. Permanent teeth affected by enamel pitting (percentage by tooth).

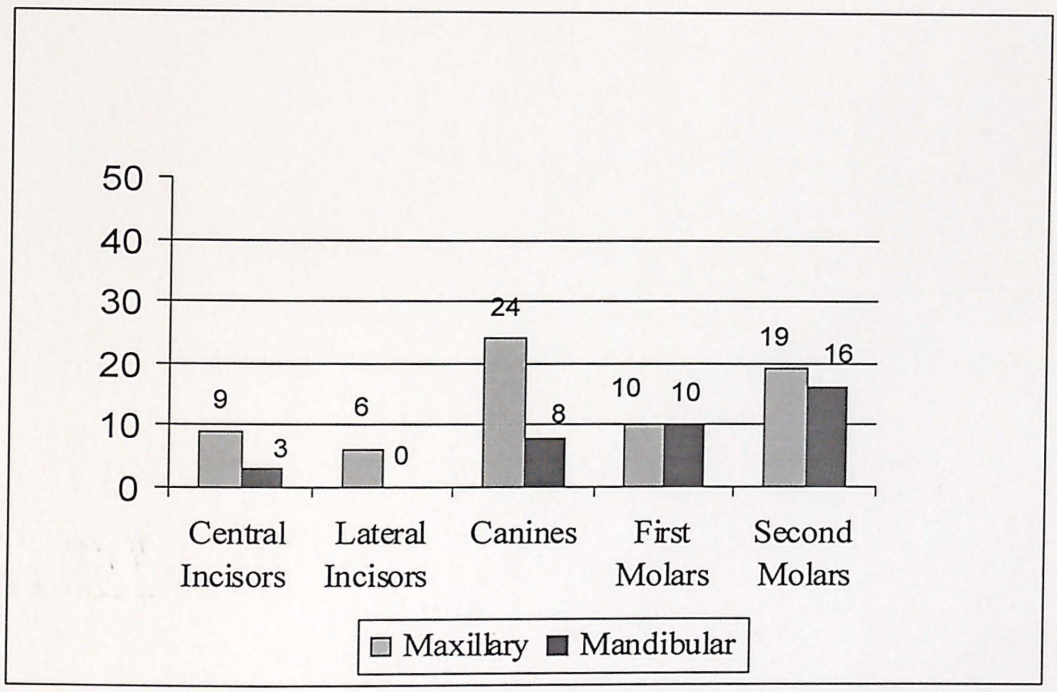
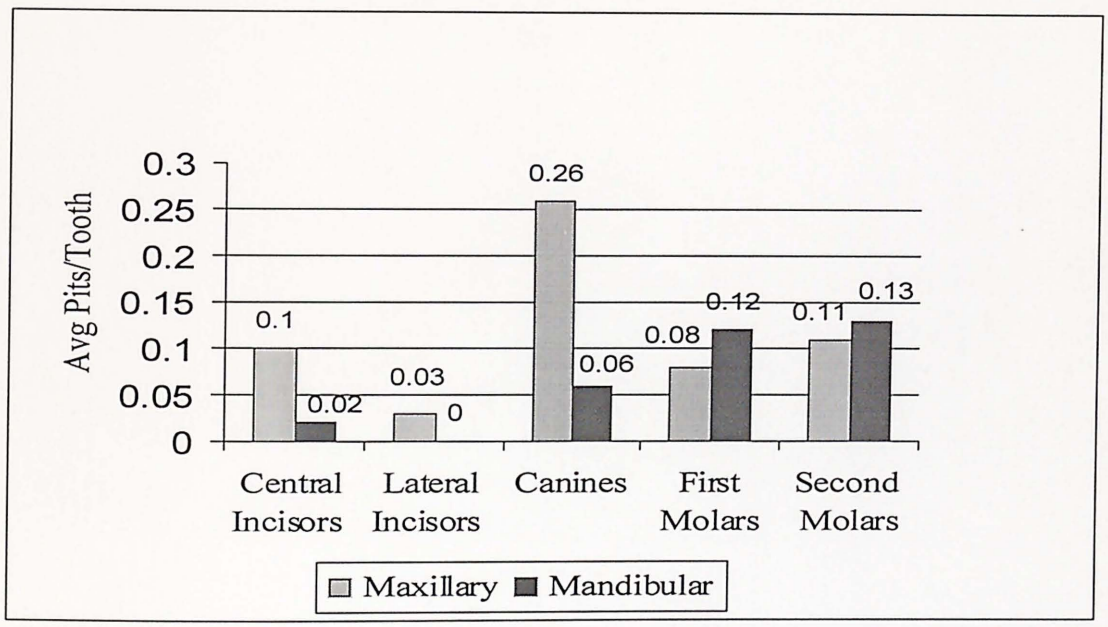
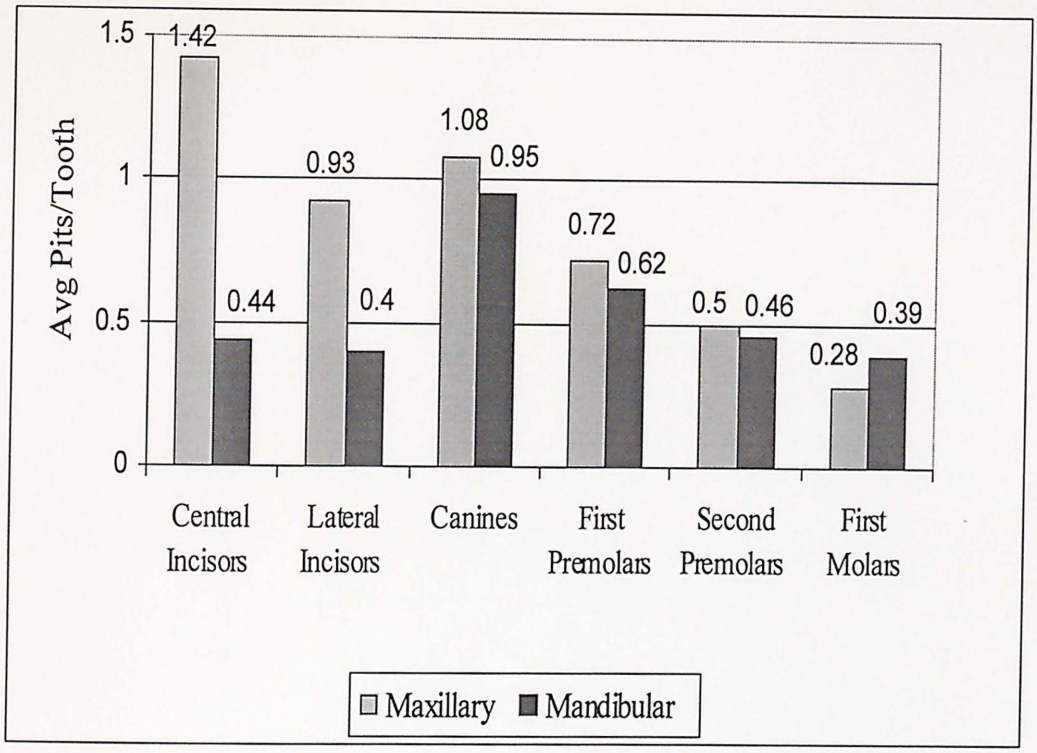


FIGURE 11. Primary teeth affected by enamel pitting (percentage by tooth).



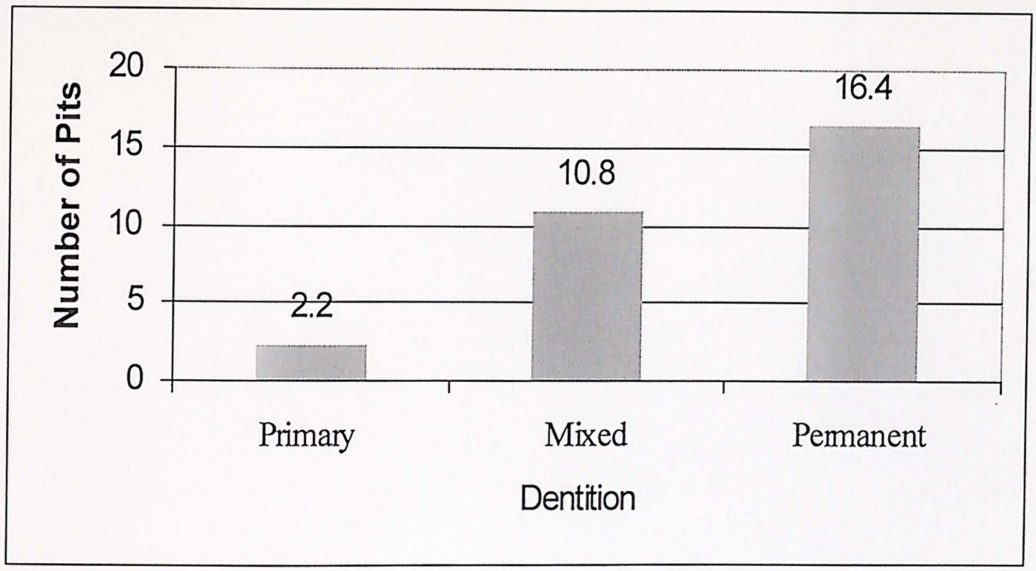
	Pitting	Central Incisors	Lateral Incisors	Canines	First Molars	Second Molars
Maxillary	Maximum	1	1	4	1	1
	Minimum	0	0	0	0	0
	Std Dev	0.3	0.11	0.63	0.24	0.25
Mandibular	Maximum	1	0	1	2	2
	Minimum	0	0	0	0	0
	Std Dev	0.08	0	0.20	0.38	0.38

FIGURE 12. Average number of enamel pits per primary tooth examined.



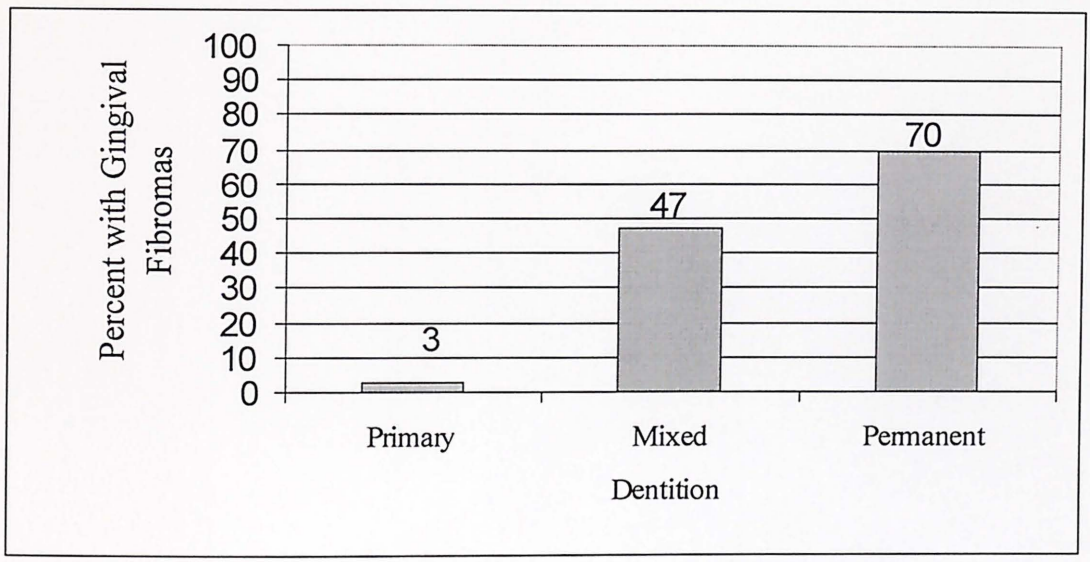
	Pitting	Central Incisors	Lateral Incisors	Canines	First Premolars	Second Premolars	First Molars
Maxillary	Maximum	8	6	5	5	3	2
	Minimum	0	0	0	0	0	0
	Std Dev	1.7	1.06	1.18	0.96	0.64	0.56
Mandibular	Maximum	3	3	5	4	3	4
	Minimum	0	0	0	0	0	0
	Std Dev	0.59	0.63	1.03	0.85	0.63	0.67

FIGURE 13. Average number of enamel pits per permanent tooth examined.



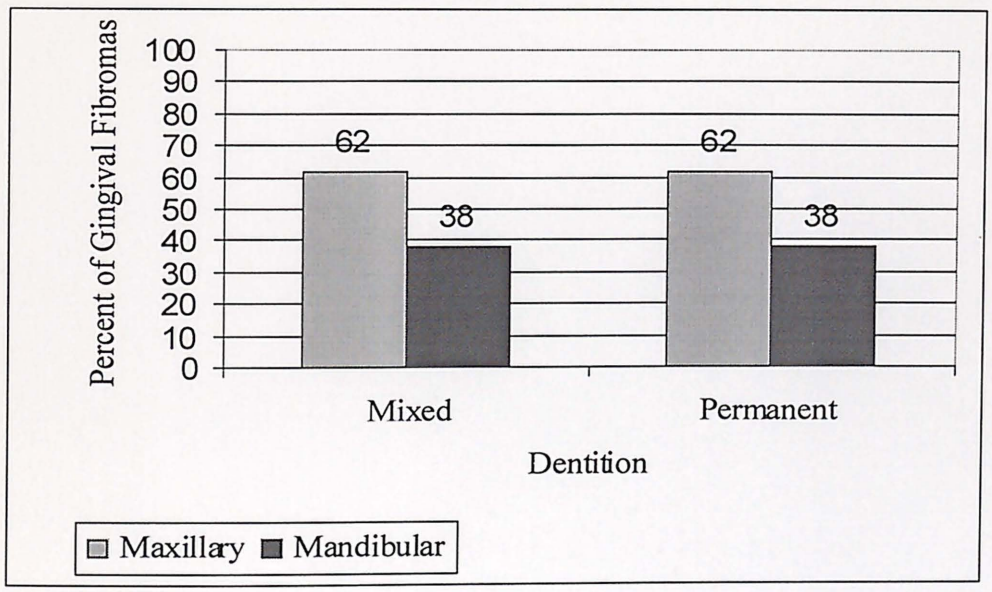
	Dentition		
	Primary	Mixed	Permanent
Patient with Enamel pits	9	17	53
Maximum/patient	4	41	68
Minimum/patient	1	1	2
Standard Deviation	1.09	10.26	13.43

FIGURE 14. Average number of enamel pits per affected patient in the primary, mixed, and permanent dentitions.



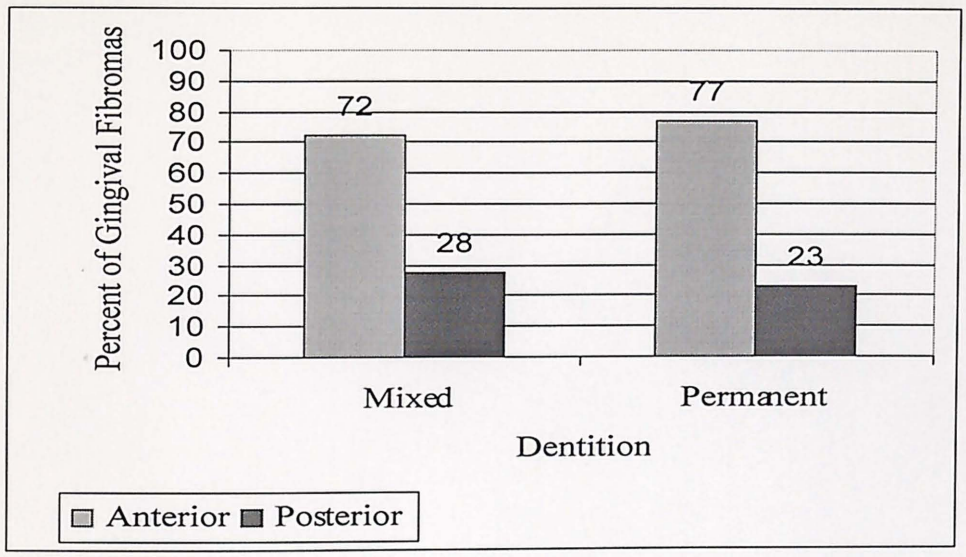
	Dentition		
	Primary	Mixed	Permanent
% of Patients with Fibromas	1	9	38
# of Patients	31	19	54

FIGURE 15. Percent of patients examined with gingival fibromas in the primary, mixed, and permanent dentitions.



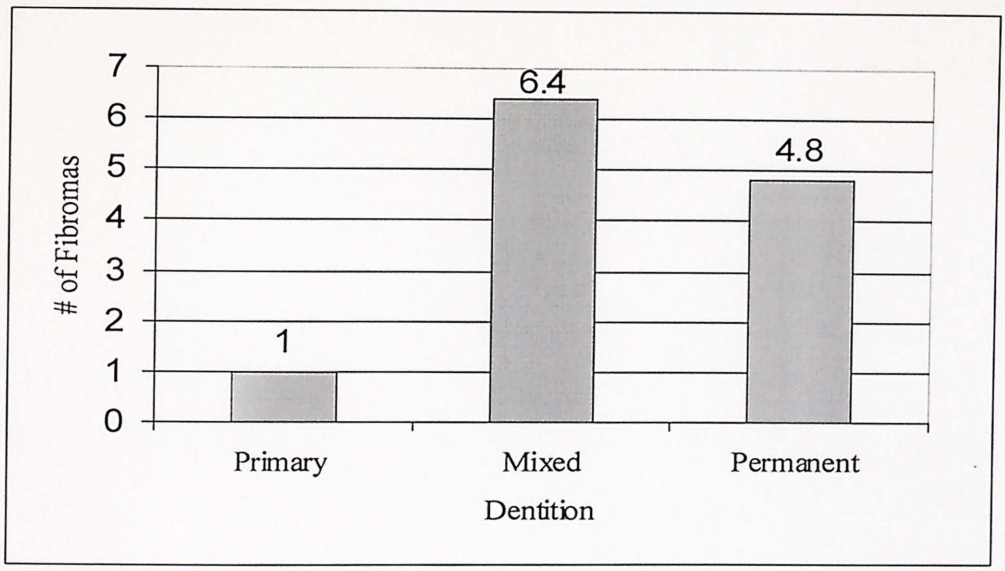
Fibromas	Dentition		
	Primary	Mixed	Permanent
Maxillary	1 (100%)	36 (62%)	112 (62%)
Mandibular	0 (0%)	22 (38%)	70 (38%)

FIGURE 16. Occurrence of gingival fibromas affecting maxillary and mandibular arches in primary and permanent teeth examined.



	Dentition		
	Primary	Mixed	Permanent
Total # of Fibromas	1	58	182
Anterior	1 (100%)	42 (72%)	140 (77%)
Posterior	0 (0%)	16 (28%)	42 (23%)

FIGURE 17. Occurrence of gingival fibromas affecting anterior and posterior arch positions in patients in the primary, mixed, and permanent dentitions.



	Dentition		
	Primary	Mixed	Permanent
# of Fibromas	1	58	182
# of Patients with fibromas	1	9	38
Maximum/patient	1	14	19
Minimum/patient	1	1	1
Standard Deviation	NA	5.47	4.22

T-Test

	N	Mean	t-value	p-value	Significant
Mixed Dentition	9	6.4	1.14	0.258	No
Permanent Dentition	38	4.8			

FIGURE 18. Average number of gingival fibromas per affected patient in the primary, mixed, and permanent dentitons. T-tests compare the means for mixed and permanent dentitions.

Table I – Part I
Enamel pitting and gingival fibromas

MR#	Date of Birth	Age In years	Gender	Dentition	Avg Pits/ Primary Tooth	%1P	%1C	Avg Pits/ Perm Tooth	%2P	%2C	#AF	#PF	T#F
650558	1/8/1991	9	Male	Mixed	1.78	100	0	2.27	76	24	0	0	0
538938	8/27/1986	13	Male	Mixed	0.5	100	0	0.78	92.85714	7.142857	10	3	13
665678	11/12/1990	9	Male	Mixed	0			0.17	50	50	1	1	2
735290	3/20/1991	9	Female	Mixed	0			0.5	90.90909	9.090909	3	1	4
653902	7/11/1989	10	Male	Mixed	0			0.08	100	0	0	0	0
747840	2/28/1994	6	Female	Mixed	0.16	100	0	0			0	0	0
947789	12/30/1989	10	Male	Mixed	0			0			0	0	0
648043	5/17/1990	9	Female	Mixed	0			0.64	100	0	0	0	0
590073	5/9/1985	13	Female	Mixed	1	0	100	1.04	76	24	0	0	0
643642	5/14/1990	8	Male	Mixed	0.42	60	40	0			0	0	0
707496	6/23/1990	10	Male	Mixed	0			0.42	80	20	4	3	7
881133	2/12/1991	9	Male	Mixed	0			0.91	80	20	0	0	0
956684	8/27/1990	9	Male	Mixed	0			0.38	100	0	0	0	0
667200	7/25/1990	9	Male	Mixed	0.25	100	0	0			11	3	14
754477	7/14/1988	11	Male	Mixed	0			0.25	100	0	1	0	1
651624	12/29/1990	9	Female	Mixed	0.17	100	0	1.5	91.66667	8.333333	1	2	3
100134	12/8/1992	7	Male	Mixed	0			0			0	0	0
538939	8/27/1986	13	Male	Mixed	0.5	100	0	0.78	92.85714	7.142857	10	3	13
825131	7/27/1991	8	Male	Mixed	0.5	100	0	0.5	50	50	1	0	1
870212	2/4/1962	38	Female	Permanent				0.54	100	0	10	4	14
766005	6/4/1969	31	Female	Permanent				0.95	90.47619	9.52381	3	2	5
100222	11/25/1964	36	Female	Permanent				0.21	100	0	1	0	1
819249	6/17/1986	14	Female	Permanent				1.63	97.4359	2.564103	2	0	2
913045	4/14/1974	26	Female	Permanent				0.68	86.66667	13.33333	1	0	1
999999	12/20/1973	25	Female	Permanent				0.46	63.63636	36.36364	10	9	19

(continued)

Table I – Part II
Enamel pitting and gingival fibromas

MR#	Date of Birth	Age In years	Gender	Dentition	Avg Pits/ Primary Tooth	%1P	%1C	Avg Pits/ Perm Tooth	%2P	%2C	#AF	#PF	T#F
367616	8/13/1980	19	Male	Permanent				0.79	100	0	0	0	0
576477	7/13/1988	11	Male	Permanent				0.13	100	0	2	0	2
999998	12/2/1955	43	Female	Permanent				0.35	100	0	1	0	1
1003601	5/7/1957	43	Female	Permanent				0.13	100	0	2	2	4
425507	2/6/1979	20	Male	Permanent				0.71	70.58824	29.41176	4	0	4
100133	10/22/1964	35	Female	Permanent				0.88	76.19048	23.80952	2	0	2
748578	3/4/1985	15	Female	Permanent				1.09	70.83333	29.16667	1	0	1
100100	5/7/1971	29	Male	Permanent				0.96	69.56522	30.43478	7	1	8
885963	4/27/1965	34	Female	Permanent				1.41	100	0	1	3	4
955338	5/24/1983	16	Male	Permanent				0.21	100	0	8	0	8
345963	3/23/1979	21	Male	Permanent				0.46	81.81818	18.18182	2	0	2
901893	9/28/1965	33	Female	Permanent				1.35	48.3871	51.6129	3	0	3
258704	4/23/1974	26	Female	Permanent				0.88	71.42857	28.57143	4	1	5
955348	1/9/1982	18	Male	Permanent				1.42	82.35294	17.64706	7	2	9
100110	9/29/1964	35	Female	Permanent				1.77	100	0	6	0	6
787714	1/18/1978	22	Male	Permanent				0.75	94.44444	5.555556	3	0	3
956892	2/24/1976	24	Female	Permanent				0.75	72.22222	27.77778	4	1	5
553907	7/29/1987	12	Female	Permanent				0.63	80	20	5	0	5
976346	11/19/1968	31	Male	Permanent				0.79	63.15789	36.84211	2	0	2
385891	9/16/1980	19	Female	Permanent				0.42	100	0	14	0	14
100006	12/21/1962	37	Female	Permanent				0.35	100	0	0	0	0
222222	8/28/1981	18	Female	Permanent				0.25	100	0	9	2	11
936854	12/2/1955	44	Female	Permanent				0.25	80	20	1	1	2
447887	8/25/1982	18	Female	Permanent				0.13	66.66667	33.33333	0	1	1
100200	3/15/1960	40	Female	Permane(nt				0.08	100	0	2	1	3

(continued)

Table I – Part III
Enamel pitting and gingival fibromas

MR#	Date of Birth	Age In years	Gender	Dentition	Avg Pits/ Primary Tooth	%1P	%1C	Avg Pits/ Perm Tooth	%2P	%2C	#AF	#PF	T#F
100005	12/25/1955	44	Female	Permanent				0.6	75	25	0	2	2
886514	12/12/1986	13	Male	Permanent				0.08	50	50	0	0	0
738440	7/6/1987	12	Male	Permanent				0.75	88.88889	11.11111	1	0	1
300635	5/16/1970	30	Female	Permanent				0.46	81.81818	18.18182	0	0	0
260096	2/3/1974	26	Male	Permanent				0.42	90	10	5	2	7
100130	1/22/1949	51	Female	Permanent				1	87.5	12.5	0	0	0
251743	10/28/1969	31	Male	Permanent				2.83	79.41176	20.58824	3	0	3
604607	6/15/1988	12	Female	Permanent				0.17	100	0	0	0	0
968031	5/28/1988	12	Male	Permanent				0.71	88.23529	11.76471	0	0	0
825130	5/16/1984	16	Female	Permanent				1.33	84.375	15.625	0	0	0
100002	2/28/1967	33	Female	Permanent				0.17	100	0	0	0	0
535368	3/25/1986	13	Female	Permanent				1.46	100	0	0		0
751954	6/1/1987	12	Female	Permanent				0.08	100	0	0	0	0
279367	7/22/1973	26	Female	Permanent				0.17	75	25	0	2	2
937450	7/17/1981	19	Male	Permanent				0.92	90.90909	9.090909	0	2	2
542204	7/15/1988	12	Female	Permanent				0.17	75	25	1	0	1
100003	8/21/1968	31	Male	Permanent				0.08	100	0	0	0	0
100004	1/7/1983	17	Female	Permanent				0.46	100	0	0	0	0
100101	2/5/1973	22	Female	Permanent				0.42	70	30	0	0	0
442009	10/7/1982	17	Female	Permanent				1.46	91.42857	8.571429	0	0	0
187859	2/26/1968	32	Female	Permanent				1.88	42.22222	57.77778	3	0	3
952798	12/28/1979	20	Female	Permanent				0.75	72.22222	27.77778	0	0	0
770763	9/1/1994	6	Female	Primary	0.1	100	0				1	0	1
816446	8/6/1994	6	Male	Primary	0						0	0	0
968603	3/14/1998	2	Female	Primary	0						0	0	0

(continued)

Table I – Part IV
Enamel pitting and gingival fibromas

MR#	Date of Birth	Age In years	Gender	Dentition	Avg Pits/ Primary Tooth	%1P	%1C	Avg Pits/ Perm Tooth	%2P	%2C	#AF	#PF	T#F
980596	8/16/1995	5	Female	Primary	0						0	0	0
926965	2/24/1998	2	Female	Primary	0						0	0	0
830937	6/24/1996	4	Male	Primary	0						0	0	0
871701	9/28/1997	2	Male	Primary	0						0	0	0
898884	10/30/1997	3	Female	Primary	0.2	100	0				0	0	0
971486	1/25/1995	5	Male	Primary	0						0	0	0
976334	12/19/1998	1	Male	Primary	0.06	0	100				0	0	0
963382	6/9/1993	7	Female	Primary	0						0	0	0
797818	9/11/1995	4	Female	Primary	0						0	0	0
798003	7/11/1995	4	Female	Primary	0						0	0	0
100001	4/16/1996	3	Female	Primary	0						0	0	0
853946	4/8/1997	3	Male	Primary	0						0	0	0
748228	2/28/1994	5	Male	Primary	0.1	100	0				0	0	0
808288	12/28/1995	4	Male	Primary	0						0	0	0
760824	5/13/1993	6	Male	Primary	0						0	0	0
768864	10/29/1994	5	Female	Primary	0						0	0	0
956669	9/25/1997	2	Female	Primary	0						0	0	0
919291	5/22/1998	2	Male	Primary	0						0	0	0
819241	12/25/1995	3	Female	Primary	0.1	100	0				0	0	0
889314	8/9/1996	3	Male	Primary	0						0	0	0
896539	6/1/1998	1	Female	Primary	0						0	0	0
735516	6/12/1993	5	Female	Primary	0.2	75	25				0	0	0
749246	2/24/1993	6	Female	Primary	0.1	100	0				0	0	0
781670	1/19/1994	4	Male	Primary	0.05	100	0				0	0	0
882846	3/15/1997	1	Male	Primary	0						0	0	0

(conitnued)

Table I – Part V
Enamel pitting and gingival fibromas

MR#	Date of Birth	Age In years	Gender	Dentition	Avg Pits/ Primary Tooth	%1P	%1C	Avg Pits/ Perm Tooth	%2P	%2C	#AF	#PF	T#F
924171	9/29/1997	2	Male	Primary	0.1	100	0				0	0	0
958384	4/22/1998	2	Male	Primary	0						0	0	0
855110	1/27/1997	2	Male	Primary	0						0	0	0

TABLE II - Part I

Phenotype Variables												
MR#	PKD	Hypertension	Cardiac	Ash Leaf	Angiofibromas	Cognitive Impairment	# of Tubers	# of AEDs used	Arrhythmias	AML	Retinal Sx	Seizure Severity
768864	No	No	No	Yes	No	Moderate		7	No	No	No	Intractable
898884						Moderate	0	0				Controllable
936854						Unaffected						Controllable
999998						Mild						
576477	No	No	No	Yes	Yes	Mild	12	1	No	Yes	Yes	Controllable
367616	No	No	No	Yes	Yes	Moderate	7	3	No	No		Controllable
667200	No	No	Yes	Yes	Yes	Severe	23	3	No	No		Controllable
797818	No	No	Yes	Yes	Yes	Mild	13	2	Yes	No	Yes	Controllable
648043	Yes	No	No	Yes	Yes	Moderate		2	No	Yes		Controllable
222222	No	No			Yes	Unaffected		3		No		No
968031	No	No	Yes			Severe		2		Yes		Intractable
279367	No	No	Yes	Yes	No	Unaffected		0	No	No	No	Controllable
665678	No	No	No	Yes	No	Moderate		2	No	No	No	Controllable
100222						Unaffected	0	0				No
955338						Mild						Controllable
535368	No	No	No	Yes	Yes	Unaffected		1	No	No		Controllable
976334						Mild						No
871701	No	No	No	Yes	No	Moderate		1	No	No	No	Controllable
956892				Yes	Yes	Mild				Yes		Controllable
748578	No	No	Yes	Yes	Yes	Severe	13	5	No	Yes	No	Controllable
913045	No	No		Yes	Yes	Moderate	14	5		Yes		Controllable
955348						Unaffected						No

(continued)

TABLE II - Part II

Phenotype Variables												
MR#	PKD	Hypertension	Cardiac	Ash Leaf	Angiofibromas	Cognitive Impairment	# of Tubers	# of AEDs used	Arrhythmias	AML	Retinal Sx	Seizure Severity
825131	No	No	No	Yes	Yes	Severe		0	Yes	Yes		Controllable
819241	Yes	No	No	Yes	No	Severe	11	0	No	No	No	Intractable
870212	Yes	No	No	Yes	Yes	Severe		0	No	Yes	No	Intractable
760824	No	No	No	Yes	Yes	Severe	22	0	No	No	No	Controllable
604607						Unaffected						Controllable
825130	No	No	Yes	Yes	Yes	Unaffected		0	No	No		No
754477	Yes	Yes	Yes	Yes	Yes	Moderate		1	No	Yes	Yes	Controllable
251743						Severe	0	0				Controllable
819249	No	No		Yes	Yes	Severe		0		Yes		Intractable
735516	No	No	Yes	Yes	Yes	Mild		0	Yes	No	No	Controllable
590073	No	No	Yes	Yes	Yes	Moderate		0	No	No	No	Controllable
385891	No	No	No	Yes	Yes	Mild		0	No	No	No	Controllable
952798												Controllable
937450	No		Yes	Yes	No	Unaffected		0	Yes	Yes		No
100005						Unaffected						No
447887	No	No	No	Yes	Yes	Unaffected		8	No	No	No	Controllable
963382						Severe						Intractable
971486						Unaffected						No
100110						Unaffected						Controllable
924171	No	No				Severe		4		No		Intractable
968603	Yes	No	Yes	Yes	No	Mild		4	No	No		Intractable
553907	No	No	No	Yes		Moderate	16	1	No	No		Controllable

(continued)

TABLE II - Part III

Phenotype Variables												
MR#	PKD	Hypertension	Cardiac	Ash Leaf	Angiofibromas	Cognitive Impairment	# of Tubers	# of AEDs used	Arrhythmias	AML	Retinal Sx	Seizure Severity
748228	No	No	Yes	Yes	No	Severe	11	0	Yes	Yes	No	Controllable
100001	No	No	No	Yes	No	Mild			No	No	No	Controllable
100003						Unaffected						No
100002						Unaffected						No
100200						Unaffected						Controllable
643642	Yes	No	Yes	Yes	No	Unaffected		0	No	Yes		No
751954	No	No	No	Yes	Yes	Moderate	22	0	No	Yes	No	Controllable
881133	No	No	No	Yes	Yes	Moderate		0	No	Yes	No	Controllable
886514						Unaffected						Controllable
980596						Mild						Controllable
919291	No	No	Yes	Yes	No	Unaffected		0	No	No		Controllable
830937	No	No	No	Yes	No	Unaffected		0	No	No	No	Controllable
300635	No	No		Yes	Yes	Unaffected		0		Yes		Controllable
855110			No	Yes	No	Unaffected		0	No			Controllable
651624	No	No		Yes	Yes	Mild	16	0	No	Yes		Controllable
653902	No	No	No	Yes	No	Unaffected		3	No	Yes		Controllable
538938	No	No	No	Yes	Yes	Unaffected	19	0	No	No		No
538939	No	No	No	Yes	Yes	Unaffected	19	0	No	No		No
976346						Moderate						Controllable
442009	Yes	No	No	Yes	Yes	Severe		0	No	Yes	No	Controllable
882846	No	No	Yes	Yes	No	Mild		1	No	No	No	Intractable
100006						Unaffected						No

(continued)

TABLE II - Part IV

Phenotype Variables												
MR#	PKD	Hyperten-sion	Cardiac	Ash Leaf	Angio-fibromas	Cognitive Impairment	# of Tubers	# of AEDs used	Arrhyth-mias	AML	Retinal Sx	Seizure Severity
816446			Yes			Unaffected		0				No
103601						Unaffected						Controllable
650558	Yes	No	Yes	Yes		Moderate	11	3	Yes	Yes	No	Controllable
542204	No	No	Yes	Yes	Yes	Moderate	3	0	No	Yes	No	Controllable
889314		No	Yes	Yes	No	Unaffected		0	No		No	Controllable
425507	Yes	No	Yes	Yes	Yes	Moderate	13	0	Yes	No	No	Controllable
956669	No	No	No	Yes		Mild			No	No		Intractable
787714	Yes	No	Yes	Yes	Yes	Moderate		0	No	No	No	Intractable
100004												No
901893		No	No	Yes	Yes	Unaffected		0	Yes	Yes	No	No
345963	Yes	No	No	Yes	Yes	Moderate		0		Yes	Yes	Controllable
766005	No	No	No	Yes	Yes	Moderate	2	4	No	No	No	Controllable
707496						Severe						Intractable
258704	No		No		Yes	Mild		0	No	Yes		No
187859	No	No	Yes	Yes	Yes	Severe		0	No	No	Yes	Intractable
747840	Yes	No	Yes	Yes	Yes	Unaffected		0	Yes	No		Controllable
749246	No	No	Yes	Yes	No	Unaffected	15	0	Yes	No	No	No
100133	No	No			Yes	Mild				Yes		Controllable
100134	No	No	Yes	Yes	No	Moderate	16	0	No	Yes		Intractable
798003	No	No	No	Yes	No	Mild		0	No	No	No	Controllable
738440	Yes	No	No	Yes	Yes	Moderate	22	0	No	Yes		Controllable
735290	No	No	Yes	Yes	Yes	Mild		0	No	No	Yes	Controllable

(continued)

TABLE II - Part V

Phenotype Variables												
MR#	PKD	Hypertension	Cardiac	Ash Leaf	Angiofibromas	Cognitive Impairment	# of Tubers	# of AEDs used	Arrhythmias	AML	Retinal Sx	Seizure Severity
260096						Severe						Controllable
958384						Unaffected						No
956684						Unaffected						No
926965	No	No	No	Yes	No	Unaffected		0	No	No		Controllable
999999	No	Yes	Yes	Yes	Yes	Unaffected		0	No	Yes	Yes	Controllable
100100		No	No	Yes	Yes	Unaffected		2	No	Yes		Controllable
808288						Moderate		0				Controllable
947789	No		Yes	Yes		Moderate			Yes	Yes		Controllable
896539		No	Yes	Yes	No	Moderate		0	No		No	Controllable
853946		No	Yes	Yes	No	Unaffected		0	No		No	No
885963	Yes	No	Yes	Yes	Yes	Unaffected		0	No	Yes		Intractable
770763	No	No	Yes	Yes	Yes	Unaffected	0	0	No	No	No	No

TABLE III Part I
Mutation/Familial vs Sporadic Disease

MR#	Familial/ Sporadic	Mutation	Mutation Type	Mutation Category
650558	Familial			
538938	Familial	2:E 33.2 4432G>C D1478H-1	Missense	Nontruncating
735290	Sporadic	2:del 4.5 Kb	Missense	Nontruncating
653902	Familial	2:E33 4271 A>G, 1424 D?G	Missense	Nontruncating
648043	Sporadic	2:4681-1 G>A	Splice	Truncating
590073	Familial	2:19, 465 kb deletion, Long PCR	Large Deletion	Truncating
643642	Familial	T1E4 342 C>A, L41I	Missense	Nontruncating
881133	Sporadic	2:4843 del TGCT GGC	Deletion	Truncating
667200		2:(E29)3411C>T,R1138X	Nonsense	Truncating
100134	Familial	2:5042 C>T, P 1675 L		
538939	Familial	2:E30 3750 C>G, Y1250X	Nonsense	Truncating
825131	Sporadic	2:E9 911G>A, W304X	Nonsense	Truncating
870212	Sporadic	2:E39 5126 C>T,P1709L-3	Missense	Nontruncating
819249			no mutation	no mutation
913045	Sporadic	2:E16 1832 G>A, R611Q	Missense	Nontruncating
999999	Familial	2:18 2098-1 G>A	Splice	Truncating
367616	Familial	2:E27, 3212 del A	Frameshift	Truncating
425507		1:I4, 432-1 G>A*	Splice	Truncating
100133	Familial	2:5042 C>T, P 1675 L	Missense	Nontruncating
748578		2:792+1 2T>A	Splice	Truncating
100100	Familial	2:18 2098-1 G>A	Splice	Truncating
885963	Familial	2:E32 3985 Ins G	Frameshift	Truncating
901893	Familial	2:E41, 5276 C>A,A17759 D-2	Missense	Nontruncating
100110	Familial			
956892		2:E9, 903-922 del	Deletion	Truncating
385891	Familial	1:E19 2713 ins A	Frameshift	Truncating
100006	Familial			
936854	Sporadic			
447887		E12 1318 G>A, 440G>S	Missense	Nontruncating
100200	Familial			
100005	Familial	1:E15.1 1744 C>T, R582X	Nonsense	Truncating
738440		2:E10 1094 3 pb del lose I&E get K	Deletion	Truncating
300635	Familial			
260096	Sporadic	2:E29 3411 C>T, R1138X	Nonsense	Truncatiing
279367	Familial	2:1795 A>G, H593R	polymorphism	Nontruncating
937450	Familial	1:E15.1 1744C>T,R582X	Nonsense	Truncating
542204	Sporadic	2:E40 5205 C>G 1735 I>M	Splice	Truncating
100004	Familial			
100101	Familial			
442009		2:885 ins C	Splice	Truncating
187859	Familial	E18 1960-61, del GG	Deletion	Truncating
952798	Familial	1:E15.1 1744 C>T, R582X	Nonsense	Truncating
770763	Sporadic	1:E17 2355 5bp del CATCD- deletion	Deletion	Truncating
816446	Familial			

(continued)

TABLE III Part II
Mutation/Familial vs Sporadic Disease

MR#	Familial/ Sporadic	Mutation	Mutation Type	Mutation Category
968603	Sporadic	2:E38, 5075, del AGGG	Deletion	Truncating
926965	Familial	2:I18 2098-1 G>A	Splice	Truncating
871701	Sporadic	1:1054 del CT	Nonsense	Truncating
898884	Sporadic			
971486	Familial			
963382	Familial			
797818		2:E22 2639 +G>C	Splice	Truncating
798003	Sporadic	2:209 del TGC	Deletion	Truncating
100001		E17 1865 G>A, 622 R>Q	Missense	Nontruncating
853946	Familial			
748228	Familial	2:E40 5228 G>A, R1743Q+1	Missense	Nontruncating
808288		2:E13 1372 C>T, R458X	Nonsense	Truncating
760824		2:5270-77+1 19 del27	Frameshift	Truncating
768864		1:E8, 954C>T, R245X	Nonsense	Truncating
919291	Familial			
819241		2:G3430 C>T, R1144X	Nonsense	Truncating
896539	Sporadic	2:E37 4854del 1BP (C) Deletion	Deletion	Truncating
735516	Familial	2:I9, 465 kb deletion, Long PCR	Large Deletion	Truncating
749246	Familial			
781670		2:1237 T>G, Y407D	Missense	Nontruncating
882846	Sporadic	2:990 C>G, Y324X	Nonsense	Truncating
855110	Familial	2:E118, 2098-1 G>A	Splice	Truncating

TABLE IV

Key to statistical testing

TABLE V

T-Test for angiomyolipomas (AML) and oral variables

	Variable	N	Mean	t-value	p-value	Significant P < 0.05
Primary Teeth	Pitting	N=25 Y=11	0.122 0.27	-1.17	0.25	No
	Pinpoint	N=11 Y=5	88.63 92	-0.23	0.82	No
	Craters	N=11 Y=5	11.36 8	0.23	0.82	No
Permanent Teeth	Pitting	N=19 Y=31	0.67 0.72	-0.34	0.73	No
	Pinpoint	N=17 Y=28	82.72 84	-0.26	0.79	No
	Craters	N=17 Y=28	17.27 15.99	0.26	0.79	No
Fibromas	Total	N=37 Y=32	2.84 2.56	-0.25	0.80	No
	Anterior	N=37 Y=32	2.08 2	0.10	0.92	No
	Posterior	N=36 Y=32	0.5 0.84	-0.96	0.34	No

TABLE VI

T-Test for polycystic kidney disease (PKD) and oral variables

	Variable	N	Mean	t-value	p-value	Significant P < 0.05
Primary Teeth	Pitting	N=29 Y=7	0.123 0.35	-1.58	0.123	No
	Pinpoint	N=12 Y=4	89.53 90	-0.3	0.97	No
	Craters	N=12 T=4	10.41 10	0.03	0.97	No
Permanent Teeth	Pitting	N=34 Y=13	0.657 0.752	-0.53	0.59	No
	Pinpoint	N=31 Y=11	82.748 91.197	-1.62	0.59	No
	Craters	N=31 Y=11	17.252 8.803	1.62	0.11	No
Fibromas	Total	N=51 Y=15	2.490 2.866	-0.28	0.78	No
	Anterior	N=51 Y=15	1.862 2.133	-0.27	0.79	No
	Posterior	N=50 N=15	0.64 0.733	-0.21	0.83	No

TABLE VII

T-Test for cardiac rhabdomyomas and oral variables

	Variable	N	Mean	t-value	p-value	Significant P < 0.05
Primary Teeth	Pitting	N=17 Y=22	0.0941 0.189	-0.86	0.39	No
	Pinpoint	N=4 Y=10	100 83.5	0.99	0.34	No
	Craters	N=4 Y=10	0 16.5	-0.99	0.34	No
Permanent Teeth	Pitting	N=23 Y=20	0.669 0.683	-0.08	0.93	No
	Pinpoint	N=23 Y=15	84.97 79.87	0.92	0.36	No
	Craters	N=23 Y=15	15.027 20.123	-0.92	0.36	No
Fibromas	Total	N=33 Y=33	3.121 1.787	1.20	0.23	No
	Anterior	N=33 Y=33	2.515 1.181	1.60	0.11	No
	Posterior	N=32 Y=33	0.625 0.606	0.05	0.95	No

TABLE VIII

T-Test for cardiac arrhythmias and oral variables

	Variable	N	Mean	t-value	p-value	Significant P < 0.05
Primary Teeth	Pitting	N=30 Y=9	0.101 0.321	-1.75	0.08	No
	Pinpoint	N=8 Y=7	82.5 96.43	-0.99	0.34	No
	Craters	N=8 Y=7	17.5 3.57	0.99	0.34	No
Permanent Teeth	Pitting	N=35 Y=7	0.675 0.821	-0.63	0.53	No
	Pinpoint	N=32 Y=5	85.57 67.177	2.39	0.02	Yes
	Craters	N=32 Y=5	14.43 32.82	-2.39	0.02	Yes
Fibromas	Total	N=52 Y=12	2.942 0.833	2.65*	0.01	Yes
	Anterior	N=52 Y=12	2.173 0.666	2.31*	0.02	Yes
	Posterior	N=51 Y=12	0.784 0.166	2.18*	0.03	Yes

* = Satterthwaite t-Test Method, variances unequal.

TABLE IX

T-Test for facial angiofibromas and oral variables

	Variable	N	Mean	t-value	p-value	Significant $P < 0.05$
Primary Teeth	Average Pitting	N=20 Y=15	0.036 0.225	-2.76	0.009	Yes
	%Pinpoint	N=4 Y=9	90 86.11	0.21	0.83	No
	%Craters	N=4 Y=9	10 13.88	-0.21	0.83	No
Permanent Teeth	Average Pitting	N=6 Y=40	0.223 0.756	-2.62	0.012	Yes
	%Pinpoint	N=4 Y=38	78.97 84.16	-0.60	0.55	No
	%Craters	N=4 Y=38	21.03 15.83	0.60	0.55	No
Fibromas	Total	N=22 Y=44	0.272 3.977	-3.32	0.0015	Yes
	Anterior	N=22 Y=44	0.045 3.068	-3.63	0.0006	Yes
	Posterior	N=22 Y=43	0.227 0.930	-1.82	0.07	No

TABLE X

T-Test for retinal lesions and oral variables*

	Variable	N	Mean	t-value	p-value	Significant P < 0.05
Primary Teeth	Pitting	N=20 Y=3	0.171 0	0.66	0.51	No
	Pinpoint	N=7 Y=0	IN	IN	IN	IN
	Craters	N=8 Y=0	IN	IN	IN	IN
Permanent Teeth	Pitting	N=17 Y=6	0.75 0.613	0.48	0.63	No
	Pinpoint	N=17 Y=6	80.28 79.76	0.06	0.95	No
	Craters	N=17 Y=6	19.71 20.23	-0.06	0.95	No
Fibromas	Total	N=33 Y=7	1.969 4.428	-1.30	0.20	No
	Anterior	N=33 Y=7	1.545 3	-1.04	0.30	No
	Posterior	N=33 Y=7	0.424 1.428	-1.46	0.15	No

*IN = insufficient numbers.

TABLE XI

T-Test for seizures vs. no seizures and oral variables

	Variable	N	Mean	t-value	p-value	Significant $P < 0.05$
Primary Teeth	Pitting	N=11 Y=39	0.152 0.118	0.33	0.744	No
	Pinpoint	N=6 Y=12	76.66 89.58	-0.78	0.448	No
	Craters	N=6 Y=12	23.33 10.41	0.78	0.448	No
Permanent Teeth	Pitting	N=17 Y=55	0.644 0.676	-0.20	0.841	No
	Pinpoint	N=16 Y=51	89.104 83.579	1.24	0.217	No
	Craters	N=16 Y=51	10.896 16.421	-1.24	0.217	No
Fibromas	Total	N=24 Y=79	2.5 2.278	0.23	0.814	No
	Anterior	N=24 Y=79	1.875 1.734	0.19	0.846	No
	Posterior	N=24 N=78	0.625 0.551	0.24	0.808	No

TABLE XII

T-Test for familial (F) vs. sporadic (S) TSC and oral variables

	Variable	N	Mean	t-value	p-value	Significant P < 0.05
Primary Teeth	Pitting	F = 18 S=11	0.255 0.072	1.25	0.223	No
	Pinpoint	F=8 S=3	79.375 100	-0.97	0.355	No
	Craters	F=8 S=3	20.625 0	0.97	0.355	No
Permanent Teeth	Pitting	F=29 S=8	0.713 0.555	0.75	0.459	No
	Pinpoint	F=27 S=8	84.822 84.7	0.02	0.985	No
	Craters	F=27 N=8	15.18 15.29	-0.02	0.986	No
Fibromas	Total	F=40 S=15	2.525 2	0.39	0.695	No
	Anterior	F=40 S=15	1.825 1.466	0.35	0.724	No
	Posterior	F=40 S=15	0.7 0.533	0.36	0.72	No

TABLE XIII

T-Test for nontruncating (Nt) vs. truncating (T) mutations and oral variables

	Variable	N	Mean	t-value	p-value	Significant P < 0.05
Primary Teeth	Pitting	Nt=7 T=17	0.152 0.155	-0.03	0.979	No
	Pinpoint	Nt=4 T=7	90 82.14	0.38	0.709	No
	Craters	Nt=4 T=7	10 17.85	-0.38	0.709	No
Permanent Teeth	Pitting	Nt=11 T=19	0.264 0.577	-0.94	0.353	No
	Pinpoint	Nt=10 T=18	84.54 78.93	0.85	0.401	No
	Craters	Nt=10 T=18	15.45 21.06	-0.85	0.401	No
Fibromas	Total	Nt=12 T=34	3.17 2.35	-0.513	0.611	No
	Anterior	Nt=12 T=34	2.42 1.74	-0.567	0.574	No
	Posterior	Nt=12 T=34	0.75 0.62	-0.242	0.588	No

TABLE XIV

Chi-square test for oral fibromas vs. systemic variables*

Variable	N	Chi square	Prob	Significant
AML	N=15 Y=12	3.31	0.0688	No
PKD	N=24 Y=8	0.1827	0.6691	No
Cardiac Rhabdomyomas	N=13 Y=13	0.5536	0.4569	No
Cardiac Arrhythmias	N=25 Y=4	0.8553	0.3551	No
Facial Angiofibromas	N=3 Y=31	18.95	<0.0001	Yes
Retinal Lesions	N=13 Y=6	4.96	0.0258	Yes

*Oral fibromas = Yes.

TABLE XV-Part I

Correlation coefficients for phenotype variables vs. oral variables

	Primary Teeth Average # of Pits	Permanent Teeth Average # of Pits			Total Number of Oral Fibromas
IQ Estimation (Pearson's Correlation)					
Correlation Coeff	-0.405*	0.019			0.178
Sig. (2-tailed)	0.050	0.932			0.285
N	24	22			38
# of Tubers (Pearson's Correlation)					
		Avg#	Pinpoint	Craters	
Correlation Coeff		0.191	0.587*	-0.587*	-0.168
Sig. (2-tailed)	0.217	0.495	0.035	0.035	0.466
N	0.522	15	13	13	21
	11				
# of AED's used (Pearson's Correlation)					
Correlation Coeff	-0.167	-0.079			-0.014
Sig. (2-tailed)	0.330	0.606			0.911
N	36	45			68
Hx of Cardiac Rhabdomyomas (Spearman's rho)					
Correlation Coeff	0.291	-0.072			-0.084
Sig. (2 tailed)	0.085	0.653			0.510
N	36	41			64
Hx of Arrhythmias (Spearman's rho)					
		Avg#	Pinpoint	Craters	
Correlation Coeff	0.408*	0.011	-0.344*	0.348*	-0.126
Sig. (2-tailed)	0.014	0.944	0.043	0.041	0.329
N	36	40	35	35	62
Presence of Angiomyolipomas (Spearman's rho)					
Correlation Coeff	0.136	0.048			0.141
Sig. (2-tailed)	0.449	0.745			0.254
N	33	48			67
PKD (Spearman's rho)					
Correlation Coeff	0.235	0.024			0.012
Sig. (2-tailed)	0.188	0.873			0.925
N	33	46			65
Hypertension (Spearman's rho)					
Correlation Coeff	Insufficient	-0.142			0.201
Sig. (2-tailed)	Numbers	0.385			0.106
N		44			66

(continued)

TABLE XV-Part II

Correlation coefficients for phenotype variables vs. oral variables

	Primary Teeth Average # of Pits	Permanent Teeth Average # of Pits			Total Number of Oral Fibromas
Retinal Lesions (Spearman's rho)					
Correlation Coeff	-0.170	-0.137			0.372*
Sig. (2-tailed)	0.462	0.543			0.020
N	21	22			39
Facial Angiofibromas (Spearman's rho)					
Correlation Coeff	0.527*	0.378*			0.514*
Sig. (2-tailed)	0.002	0.011			0.000
N	32	44			64
Primary Teeth Average # of Pits (Pearson's Correlation)					
Correlation Coeff	NA	NA			0.177
Sig. (2-tailed)					0.239
N					46
Permanent Teeth Average # of Pits (Pearson's Correlation)					
Correlation Coeff	NA	NA			-0.008
Sig. (2-tailed)					0.954
N					60
Permanent Teeth Percent Pinpoint Pits (Pearson's Correlation)					
		Avg#	Pits	Craters	
Correlation Coeff	NA	NA	NA	-1.00**	0.121
Sig. (2-tailed)				<0.0001	0.655
N				68	16
Total Number of Oral Fibromas (Pearson's Correlation)					
Correlation Coeff	0.177	-0.008			NA
Sig. (2-tailed)	0.239	0.954			
N	46	60			
Familial vs. Sporadic Disease					
Correlation Coeff	-0.160	-0.099			-0.004
Sig. (2-tailed)	0.416	0.595			0.980
N	28	31			50
Truncating vs. Nontruncating Mutations					
Correlation Coeff	0.257	-0.276			0.117
Sig. (2-tailed)	0.215	0.147			0.439
N	25	29			46

TABLE XVI

Analysis of variance testing (ANOVA) for seizure severity and cognitive impairment vs. oral variables

		Seizure Severity			Cognitive Impairment		
	Variable	F-Value	p-Value	Significant	F-Value	p-Value	Significant
Primary Teeth	Pitting	0.62	0.539	No	0.78	0.514	No
	Pinpoint	0.40	0.6773	No	1.14	0.366	No
	Craters	0.40	0.6773	No	1.14	0.366	No
Permanent Teeth	Pitting	0.66	0.512	No	1.70	0.176	No
	Pinpoint	1.12	0.332	No	0.71	0.549	No
	Craters	1.12	0.332	No	0.71	0.549	No
Fibromas	Total	0.29	0.747	No	1.63	0.188	No
	Anterior	0.13	0.879	No	1.51	0.216	No
	Posterior	0.66	0.521	No	2.36	0.077	No

TABLE XVII

Key to summary of statistical tests

	Table	Page
Seizure Activity Number of Tubers # of AED's IQ Estimation Cognitive Impairment	XVIII-Part I	89
Angiomyolipomas (AML) Poloycystic Kidney Disease (PKD) Cardiac Rhabdomyomas Cardiac Arrhythmias	XVIII-Part II	90
Facial Angiofibromas Retinal Lesions Hypertension	XVIII-Part III	91
Familial vs. Sporadic Disease Truncating vs. Nontruncating Mutations	XIX	92

TABLE XVIII-Part I

Summary chart of statistical tests for oral findings vs. phenotype variables

		Seizure Activity Severity		# of Tubers	# of AED's	IQ Estimation	Cognitive Impairment
		t-Test	ANOVA	Pearsons Correlation	Pearsons Correlation	Pearsons Correlation	ANOVA
		Sig.	Sig.	+/-	+/-	+/-	Sig
Primary Teeth	Average Pitting	No	No	No	No	Negative	No
	Pinpoint	No	No	No	No	No	No
	Craters	No	No	No	No	No	No
Permanent Teeth	Average Pitting	No	No	No	No	No	No
	Pinpoint	No	No	Positive	No	No	No
	Craters	No	No	Negative	No	No	No
Fibromas	Total	No	No	No	No	No	No
	Anterior	No	No	No	No	No	No
	Posterior	No	No	No	No	No	No

TABLE XVIII-Part II

Summary chart of statistical tests for oral findings vs. phenotype variables

		Angiomyolipo-mas (AML)			Polycystic Kidney Disease (PKD)			Cardiac Rhabdomyomas			Cardiac Arrhythmias		
		t-Test	Spearman's rho	Chi-Square	t-Test	Spearman's rho	Chi-Square	t-Test	Spearman's rho	Chi-Square	t-Test	Spearman's rho	Chi Square
		Sig	+/-	Sig	Sig	+/-	Sig	Sig	+/-	Sig	Sig	+/-	Sig
Primary Teeth	Average Pitting	No	No	NA	No	No	NA	No	No	NA	No	Posi-tive	NA
	Pinpoint	No	No	NA	No	No	NA	No	No	NA	No	No	NA
	Craters	No	No	NA	No	No	NA	No	No	NA	No	No	NA
Permanent Teeth	Average Pitting	No	No	NA	No	No	NA	No	No	NA	No	No	NA
	Pinpoint	No	No	NA	No	No	NA	No	No	NA	Yes	Neg-ative	NA
	Craters	No	No	NA	No	No	NA	No	No	NA	Yes	Posi-tive	NA
Fibromas	Total	No	No	No	No	No	No	No	No	No	Yes	No	No
	Anterior	No	No	-	No	No	-	No	No	-	Yes	No	-
	Posterior	No	No	-	No	No	-	No	No	-	Yes	No	-

NA = Test not applicable.
\- = Test not performed.

TABLE XVIII-Part III

Summary chart of statistical tests for oral findings vs. phenotype variables

		Facial Angiofibromas			Retinal Lesions			Hypertension	
		t-test	Spearman's rho	Chi-Square	t-test	Spearman's rho	Chi-Square	Spearman's rho	Chi-Square
		Sig	+/-	Sig	Sig	+/-	Sig	+/-	Sig
Primary Teeth	Average Pitting	Yes	Positive	NA	No	No	NA	IN	NA
	Pinpoint	No	No	NA	IN	No	NA	IN	IN
	Craters	No	No	NA	IN	No	NA	IN	IN
Permanent Teeth	Average Pitting	Yes	Positive	NA	No	No	NA	No	NA
	Pinpoint	No	No	NA	No	No	NA	No	NA
	Craters	No	No	NA	No	No	NA	No	NA
Fibromas	Total	Yes	Positive	Yes	No	Positive	Yes	No	-
	Anterior	Yes	No	-	No	No	-	No	-
	Posterior	No	No	-	No	No	-	No	-

IN = Insufficient numbers to test.
NA = Test not applicable.
- = Test not performed

TABLE XIX

Summary chart of statistical tests for oral findings vs. TSC familial and sporadic disease and mutation type

		Familial vs. Sporadic Disease		Truncating vs. Nontruncating Mutations	
		t-test Sig	Spearman's rho	t-test Sig	Spearman's rho
Primary Teeth	Average Pitting	No	No	No	No
	Pinpoint	No	No	No	No
	Craters	No	No	No	No
Permanent Teeth	Average Pitting	No	No	No	No
	Pinpoint	No	No	No	No
	Craters	No	No	No	No
Fibromas	Total	No	No	No	No
	Anterior	No	No	No	No
	Posterior	No	No	No	No

DISCUSSION

According to the revised diagnostic criteria as developed at the Tuberous Sclerosis Consensus Conference in 1998, to establish a definitive diagnosis of TSC, if a patient has only one major feature, two additional minor features are required for diagnosis.[16] The diagnostic criteria for establishing a definitive diagnosis of tuberous sclerosis include multiple randomly distributed enamel pits as well as gingival fibromas, both as minor features of the disease. Considering that the majority if not all adult patients with a definitive diagnosis of TSC have been confirmed as having random enamel pitting by several investigators, [34-37] and reconfirmed by this investigation, along with a high prevalence of gingival fibromas, these clinical findings take on significant importance.

This study found pitting in 29 percent of patients between 1 and 6 years of age, in 90 percent between 6 and 13 years of age, and in 100 percent of patients in the permanent dentition. The pits were randomly distributed and without contralateral symmetry. Additionally, there was a very significant negative correlation between the percent of pinpoint sized pits and percent of crater sized pits affecting the permanent dentition. There was a strong tendency for these patients to have either a predominance of pinpoint sized pits or crater sized pits, but not both. There was also a tendency for greater pitting in the maxillary permanent central incisors (78 percent) than in other teeth. This finding is significant in that examination of these teeth after dye stain is a bit easier than examining mandibular teeth or teeth positioned more posterior in the arch, especially in cognitively impaired individuals. Because of the logistics associated with the clinical

examination of these patients in many centers, obtaining a dental examination by a dentist may be difficult if not impossible to arrange. Knowing that the majority of pitting occurs in the maxillary anterior region of the mouth should aid non-dentally trained individuals in surveying the dentition for enamel pitting.

It is important to remember that enamel pitting is a form of hypoplasia and is not pathognomonic of tuberous sclerosis. There are forms of enamel pitting associated with other conditions that must be considered when completing an oral evaluation. Individuals responsible for the oral evaluation of these patients should be well versed in the etiologies and appearances of various types of enamel pitting.

In this investigation, there were only a few patients with oral fibrous lesions in areas other than the gingiva. One patient displayed lesions on the mandibular labial mucosa, another on the buccal mucosa bilaterally, while one patient had lesions on the dorsum of the tongue. Because of the infrequent finding of nongingival oral lesions, these lesions were not tabulated for statistical purposes.

Gingival fibromas are not commonly seen in the general population. These lesions must be differentiated from gingival hyperplasia secondary to drug use (e.g. phenytoin, cyclosporin, calcium channel blocking agents), from hereditary gingival fibromatosis, from fibromatoses as an aggressive infiltrative connective tissue lesion,[71] from infective gingival pathology, and from normal variations in gingival anatomy. Each has a characteristic appearance making differentiation fairly straight forward; however, at times, trying to discern among various etiologies can be a challenge and ultimately is subjective. There were very few instances among the study subjects that identifying

TSC-related gingival fibrosis was questionable. Again, as with evaluating enamel pitting, the examiner should be well-educated in the differential diagnosis of gingival lesions.

Many of the manifestations of tuberous sclerosis are age-dependent. Facial angiofibromas, ungual fibromas, renal angiomyolipomas appear later in childhood or early adulthood,[33] while cardiac rhabdomyomas are frequently seen in infancy and tend to regress with age. Both enamel pitting and gingival fibromas are also age dependent. The height of enamel pitting occurs after the transition from the mixed dentition to the permanent dentition, and gingival fibromas are seen most frequently after 10 years of age.

There were few significant findings relating the degree and severity of enamel pitting to other physical findings of TSC, and none of the correlations were particularly strong. Enamel pitting was unrelated to seizure activity, number of antiepileptic drugs taken, cognitive impairment, angiomyolipomas, polycystic kidney disease, cardiac rhabdomyomas, retinal lesions or hypertension. In subjects with affected primary teeth, a greater degree of pitting was seen among patients with a lower estimation of IQ score. The same relations was not seen with permanent teeth, however. There was a positive relationship between the degree of pitting in both primary and permanent teeth with the presence of facial angiofibromas. The only relation between pit severity and disease variables was seen with pinpoint-sized pits in the permanent teeth and the total number of cortical tubers. The degree of pinpoint pits increased along with the total number of tubers. Unexpectedly, the reverse was true for crater-sized pits. Most of these relations are not surprising, considering the ectodermal origin of the involved tissues and that tuberous sclerosis is in fact a neurocutaneous disease.

In relating gingival fibromas with other physical findings, Lygikadis reported that large gingival fibromas affected more severely TSC-affected patients.[43] The article did not, however, define the criteria for severely affected TSC patients. This investigation failed to find meaningful associations between gingival fibromas and other symptoms associated with severe disease. As with enamel pitting, there was a significant positive correlation, however, with the appearance of gingival fibromas (regardless of size) and the appearance of facial angiofibromas. From preliminary unreported data gathered at this institution, there does not appear to be a relation between severity of facial angiofibromas and overall severity of TSC disease. There was a positive, but less significant, correlation between gingival fibromas and retinal lesions. Because of limited data, retinal lesions were all grouped into one category and not broken down into specific type. There were no other significant findings relating gingival fibromas to other TSC disease variables.

Two-thirds of individuals with TSC are thought to occur from sporadic mutations with over 80 percent at the TSC2 locus.[4] In familial cases, there is a more equal distribution between TSC1 and TSC2 mutations. Of the 53 subjects where inheritance had been established, 72 percent were determined to be familial. This discrepancy may reflect patient sampling as well as the ability of our clinic coordinator to successfully recruit family members for examination. This may also suggest that more people could be affected with familial TSC than originally thought. It has been reported that familial TSC individuals are less severely affected than those with sporadic TSC.[4] This is certainly true regarding cognitive ability and may relate to better reproductive fitness

among familial TSC. In evaluating enamel pitting and gingival fibromas however, there were not distinctions that could be made between familial versus sporadic TSC.

In regard to genotype-phenotype observations, only patients with TSC2 mutations were included due to the small number of patients with TSC1 mutations. Out of 104 study subjects, only 6 were associated with TSC1 mutations. This number is clearly reflective of sampling characteristics and is not an accurate representation of the TSC population as a whole. Additionally, because of a limited number of each type of mutation, mutations were grouped into one of two categories. Those mutations that resulted in a stop codon leading to premature termination of tuberin (small deletions or insertions, nonsense, splice site, and frameshift mutations) were classified as truncating mutations. Large genomic deletions were also included in this category because the effect is essentially the same as truncation. Mutations that did not lead to premature termination of tuberin (missense mutations and in-frame deletions) were classified as nontruncating mutations. There were no TSC2 genotype-phenotype correlations found with enamel pitting and/or gingival fibromas. These results were not surprising considering the tremendous variability in expression of the disease. This parallels other investigations that have not found TSC2 genotype-phenotype correlations with other disease variables.[52]

Future research regarding oral manifestations and the relationship to overall TSC disease is needed. Additional studies should concentrate on 1) examining a large enough patient pool to correlate oral findings in TSC1 and TSC2 individuals, 2) cohort studies to examine the changes in oral symptoms over time in individuals, 3) including better imaging of the maxillary and mandibular complex using panoramic radiographs, CT or

MRI scans to evaluate for the occurrance of intraosseous fibrous lesions as reported by Damm,[45] and finally, 4) animal studies to investigate the process of enamel formation and the role of the tuberin and hamartin on ameloblast function.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to study the incidence of enamel pitting and gingival fibromas in patients with a definitive diagnosis of tuberous sclerosis complex and relate these findings to other symptoms of TSC, to familial and sporadic disease, and specific TSC2 mutations. There have been no reports relating oral findings to these specific variables.

Subjects were recruited from the Tuberous Sclerosis Clinic at Children's Hospital Medical Center, Cincinnati, Ohio and all had a definitive diagnosis of tuberous sclerosis. A total of 104 patients ranging in age from 1 to 51 years of age were examined in the neurology clinic for the presence of enamel pitting and gingival fibromas. Subjects initially either brushed their own teeth or had their teeth brushed to remove existing plaque. The dentition was then stained with Trace disclosing solution (Red dye #28) using a 6 inch cotton swab and examined using a dental explorer and lighted mirror (DenLite by WelchAllyn). A standardized examination form was used for data collection (see Appendix I). Data collected included the total number and location of pits per tooth, size of pits, and location and number of gingival fibromas. The data were then entered into databases for tabulation and analysis.

Patients participating in the study already had extensive testing as part of a larger IRB approved investigation at Children's Hospital Medical Center entitled "Genotype/Phenotype Analysis of Individuals with Tuberous Sclerosis and Their Family Members". Test results made available for this investigation included MRI scans of the brain, echocardiography, renal ultrasound, neuropsychological assessments, and results of

retinal examinations. All study subjects had one blood sample taken for DNA extraction that was completed at Children's Hospital in Cincinnati. The DNA samples were then sent to Brigham and Women's Hospital in Boston, Massachusets for mutational analysis of either of the tuberous sclerosis genes. These results were then made available to investigators at Children's Hospital in Cincinnati. Based upon this information along with an evaluation by a geneticist, mutations were classified as being either familial or sporadic.

In evaluating the characteristics of enamel pitting, the majority of pits were pinpoint sized affecting the maxillary more often than the mandibular arch and most of the pits were anterior to the maxillary canine. The maxillary central incisor was the most often affected permanent tooth, while the maxillary canine was the most often affected primary tooth. Patients also had a tendency for a predominance of either pinpoint sized pits or crater sized pits.

The study found enamel pitting in 29% of patients between 1 and 6 years of age, in 90% between 6 and 13 years of age, and in 100 percent of patients in the permanent dentition. Because of the small number of patients under the age of six that displayed enamel pitting, its usefulness as a minor feature in the diagnosis of TSC is more limited.

The overwhelming majority of oral fibromas appeared on the maxillary anterior gingiva affecting the interdental papilla. Gingival fibromas were less likely to be seen in children under the age of six, and seemed to significantly increase in numbers after 10 years of age. In this study, 47% of individuals in the mixed dentition had gingival fibromas while 70% of individuals in the permanent dentition were affected.

There were very few significant findings relating the degree and/or severity of enamel pitting or gingival fibromas to other physical findings of TSC and none of the correlations were particularly strong. There was a positive correlation between the average number of pits in primary teeth with the presence of cardiac arrhythmias as well as for those individuals with a tendency toward crater sized pitting in the permanent teeth. There were also significant positive relationships between the appearance of facial angiofibromas with the average number of enamel pits in primary and permanent teeth, as well as with the presence of gingival fibromas. The only other significant finding was a positive correlation between the total number of gingival fibromas and retinal lesions.

In comparing oral findings to sporadic and familial TSC disease, no distinctions were evident between the two groups. Likewise, there were no TSC2 genotype-phenotype correlations found with enamel pitting or gingival fibromas.

Despite the lack of significant genotype-phenotype correlations with oral findings, enamel pitting and gingival fibromas are important features of the disease. Together, the combination of enamel pitting along with gingival fibromas, as minor features of TSC, should significantly raise the suspicion level regarding tuberous sclerosis as a diagnosis. Scanning the dentition and gingiva is noninvasive, inexpensive, easy to learn, and should be included in evaluating all patients suspected of a diagnosis of tuberous sclerosis.

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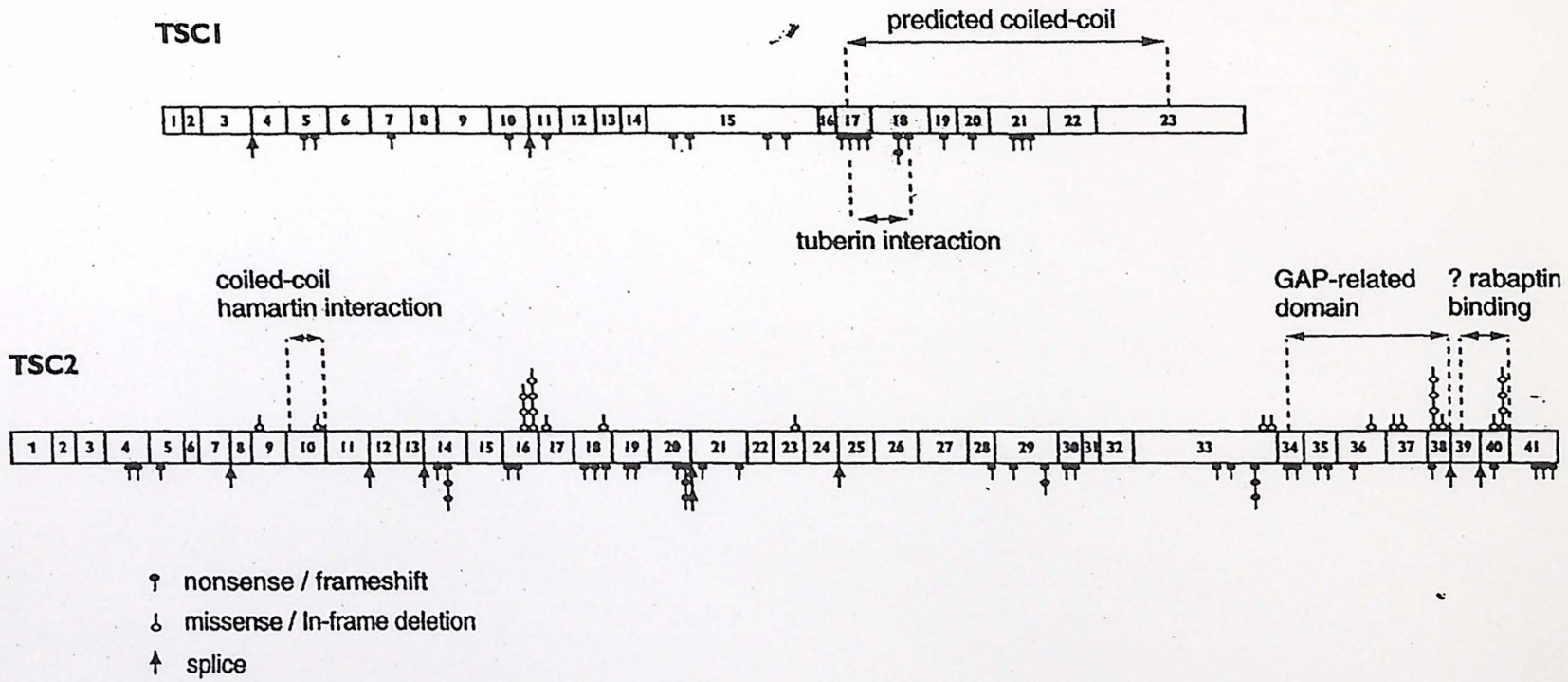
APPENDIX

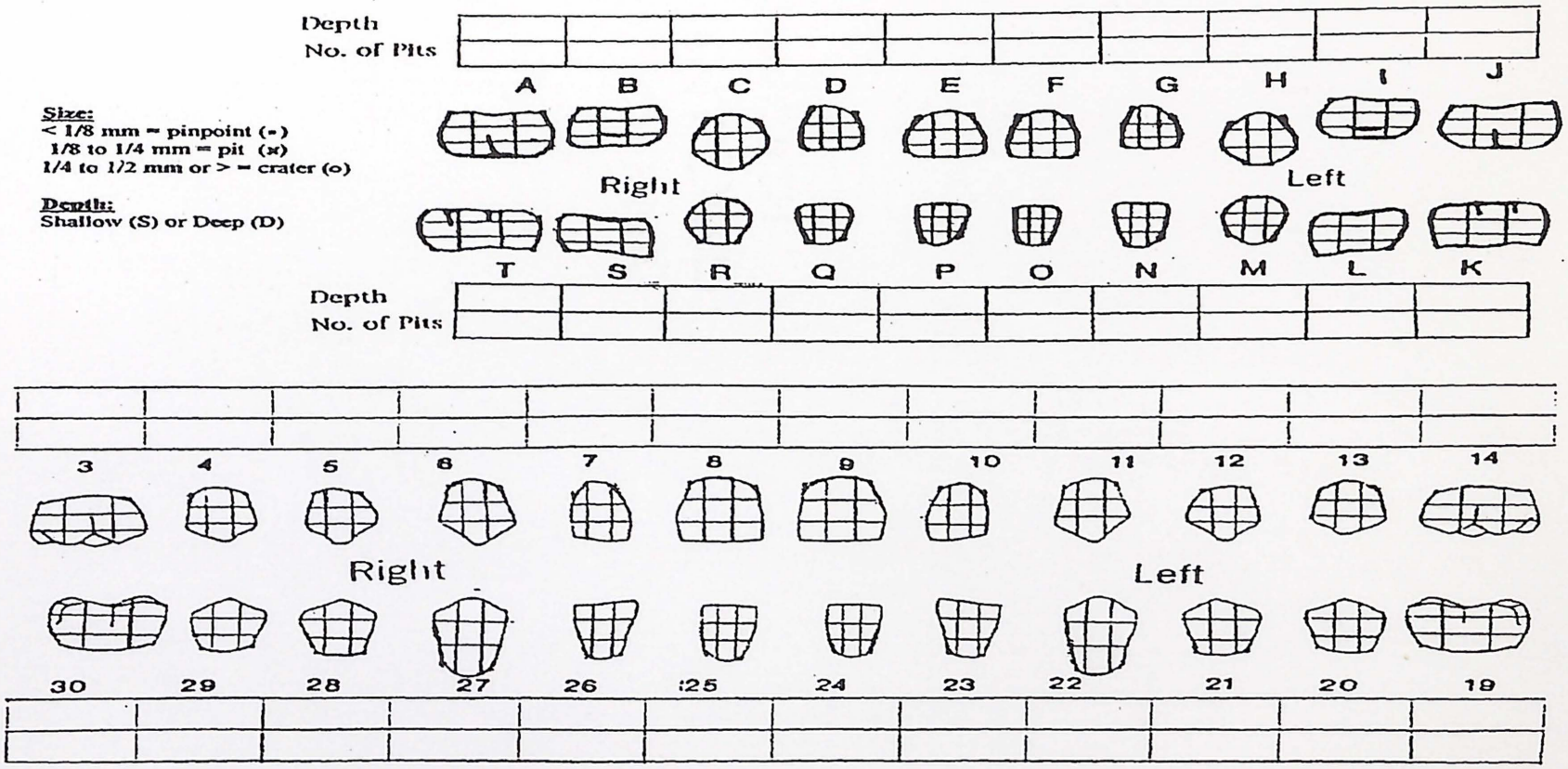
APPENDIX 1

Diagram of TSC1 and TSC2 genes showing exons and mutation spots



APPENDIX 2

Standardized Examination Form for Charting Enamel Pitting
And Gingival Fibromas



ABSTRACT

TUBEROUS SCLEROSIS-ASSOCIATED ENAMEL PITTING AND GINGIVAL FIBROMAS: FAMILIAL VS. SPORADIC DISEASE; GENOTYPE-PHENOTYPE CORRELATIONS

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The purpose of this investigation was to study the incidence of enamel pitting and gingival fibromas in patients with tuberous sclerosis complex (TSC) and relate these findings to other physical findings of TSC, to sporadic and familial disease, and to specific TSC2 mutations.

Methods: A total of 104 patients between 1 and 51 years of age were examined for enamel pits and gingival fibromas. All study subjects had a definitive diagnosis of TSC and were participants in a related study that provided results from MRI scans of the brain, echocardiography, renal ultrasound, neuropsychological assessments, and retinal examinations. Blood samples were obtained from each participant for DNA extraction and subsequent TSC mutational analysis.

Results: Enamel pitting was seen in 29% of patients between 1 and 6 years of age, in 90% between 6 and 13 years of age, and in 100% of patients in the permanent

dentition. The majority of the pits were pinpoint sized and primarily affected the maxillary anterior arch. The maxillary central incisor was the most often affected permanent tooth and the maxillary canine was the most often affected primary tooth. Gingival fibromas were apparent in 47% of subjects in the mixed dentition and in 70% of subjects in the permanent dentition. Only one patient out of 31 in the primary dentition had a gingival fibroma. The majority of fibromas affected the interdental papilla of the maxillary anterior arch. There were few significant findings relating the degree and/or severity of enamel pitting and/or gingival fibromas to other physical findings of TSC. Enamel pitting in primary as well as permanent teeth were found to be strongly related to the presence of facial angiofibromas and a somewhat weaker association was seen with cardiac arrhythmias. Gingival fibromas were strongly related to the presence of facial angiofibromas and more weakly related to retinal lesions. There were no distinctions apparent between oral findings in sporadic and familial TSC nor were there any genotype-phenotype correlations between oral findings and TSC2 mutations.

Conclusion: The combination of enamel pitting and gingival fibromas, as minor features of TSC, should raise the suspicion level regarding tuberous sclerosis as a diagnosis. Both are important minor features frequently seen which may help in establishing a definitive diagnosis. Scanning the dentition and gingiva is noninvasive, is inexpensive, and should be included in evaluating all patients suspect of a diagnosis of tuberous sclerosis.

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